PUTATIVE PHEROMONES IN THE URINE OF MALE MOOSE:

EVOLUTION OF HONEST ADVERTISEMENT?

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ABSTRACT

I tested hypotheses about how olfactory communication is related to mating behavior in Alaskan moose *(Alces alces gigas)*. Males dig rutting pits where urine is deposited to which females strongly respond. Consequently, male urine may contain primer pheromones that synchronize estrus of females. Urine samples were collected from captive moose on the Kenai Peninsula, Alaska. Samples included those from the mating season and from the nonrutting period for two adult males, one yearling male, and one male and one female calf. After pH adjustment, samples were extracted with methylene chloride to yield 3 fractions (acidic, neutral, and basic), which were analyzed by gas chromatography-mass spectrometry. Putative pheromones include unsaturated alcohols and homologs of tetrahydro-6-methyl pyranone, and 2-nonen-4-one. I hypothesize that these compounds are related to hypophagia and catabolism of body reserves by rutting males, and thereby provide an honest advertisement of body condition in moose.

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INTRODUCTION

Olfactory communication plays a major role in the behavior and ecology of mammals (Ewer 1968). Pheromones are key olfactory signals important in intra- and interspecific identification (Passanisi and MacDonald 1990), and territorial marking (Gilbert 1973, Kitchen 1974, Gosling 1987, Johansson et al. 1996, Massei and Bowyer 1999), although many mammals that scent mark are not territorial (Ralls 1971). Thus, the function of scent marking goes well beyond the delineation of territorial boundaries (Leuthold 1977, Bowyer et al. 1998b). For instance, pheromones are thought to play a fundamental role in alarm systems (Müeller-Schwarze 1969, Booth and Signoret 1992), predator-prey relationships (Mattina et al. 1991, Young 1993), maternal-infant bonds (Poindron et al. 1988, Poran et al. 1993), social structure and organization (Passanisi and MacDonald 1990), and sexual behavior (Alteri and Müeller-Schwarze 1980, Menzies et al. 1992, Rasmussen et al. 1997). Considerable evidence links olfaction and reproduction among ungulates (Watson and Radford 1960, Fraser 1968, Coblentz 1976, Bakke and Figenschou 1990, Miquelle 1991, Booth and Signoret 1992). Studies on domestic pigs and sheep have provided detailed information on olfactory mechanisms in ungulate reproduction. Pheromones of these two species have been identified and related to specific behavioral and physiological changes (Booth and Signoret 1992). Indeed, the role of chemical communication in the reproductive behavior of ungulates, especially the behaviors associated with scent marking by males has been studied for many species (Coblentz 1976).

During the mating season (rut), male urine provides an important chemical cue thought to relay information to conspecifics (McCullough 1969, Altieri and Müeller-Schwarze 1980, Izard and Vandenbergh 1982, Bakke and Figenschou 1990, Miquelle 1991). Scent-urination by ungulates may serve at least two functions: mediating aggressive interactions between males, and facilitating male-female interactions. Scent-urination directed at other males in species such as Thomson's gazelles *(Gazella thomsoni)* (Estes

1967), blackbuck antelope *(Antilope cervicapra)* (Schaller 1967), springbok *(Antidorcas marsupialis)* (David 1973) may be used for territorial marking. Scent-urination in bontebok *(Damaliscus dorcas dorcas)* (David 1973) and wildebeest *(Connochaetes taurinus)* (Estes 1969) is directed at other males as a part of a ritualized challenge. McCullough (1969) and Bowyer and Kitchen (1987) noted that scent-urination in elk *(Cervus elaphus)* occurred primarily in dominance interactions between adult males, and hypothesized that pheromones contained in urine might advertise physical condition of males. Such an olfactory cue, however, also might be involved in male-female interactions (Coblentz 1976, Bowyer and Kitchen 1987). Scent-urination by males directed at females in species such as cows, (Izard and Vandenbergh 1982), sheep (Watson and Radford 1960), feral goats (Coblentz 1976), and pigs (Poilikarpova 1945) may serve to accelerate the onset of puberty, or synchronize and induce estrus and ovulation.

In cervids, scent-urination during the mating season has been well documented. Male reindeer *(Rangifer tarandus)* scent mark by urinating on their hind feet and tramping the ground (Espmark 1964, Mossing and Damber 1981, Kojola 1991). Female reindeer exhibit an interest in the male urine deposited by rubbing against the ground, a behavior that resembles the males rubbing against urine patches (Espmark 1964). Mule deer *(Odocoileus hemionus)* also rub-urinate (Bowyer 1986), as do white-tailed deer *(Odocoileus virginianus)* (Marchinton and Hirth 1984). Caribou *(Rangifer tarandus groenlandicus)* likewise scent-urinate (Lent 1965). Some cervids such as elk *(Cervus elaphus)*, moose (*Alces alces*), and fallow deer *(Dama dama)* dig rutting pits or wallows in which urine is deposited (McCullough 1969, Bowyer and Kitchen 1987, Miquelle 1991, Massei and Bowyer 1999).

Detailed descriptions of rutting pits and the associated behaviors of moose have been provided previously (Miquelle 1991). Generally during rut, adult male moose paw a shallow depression (pit) in the ground, and urinate in the pit. Urine excreted during rut has a strong, pungent, and unique odor. These cervids impregnate their pelage with the scent by splashing urine and mud onto themselves using their front hooves, slapping urine with the underside of their antlers, and lying (wallowing) in the pit (Miquelle 1991). Females also wallow in these pits and impregnate their pelage with urine deposited by adult males (Miquelle and Van Ballenberghe 1985). Male moose attract females to their pits by scent marking with their urine, or later by scent marking (rubbing) trees (Bowyer et al. 1994).

Scent-urination in adult male moose is likely directed more towards females than males because that behavior is not temporally correlated with aggressive interactions between males, and female moose are strongly attracted to the urine of rutting males (Miquelle 1990). Unlike many other cervids, female moose impregnate their pelage by wallowing in freshly made pits. Furthermore, females exhibit a high level of antagonism towards other females while attempting to obtain access to pits (Miquelle and Van Ballenberghe 1985, Miquelle 1990). Not only are female moose interested in the urine deposited in rutting pits, but so are sub-adult males that do not scent-urinate, but presumably attempt to obtain the odor of dominants and gain the benefits of pits by wallowing in them (Miquelle and Van Ballenberghe 1985, Miquelle 1990). Those behaviors indicate that the urine of male moose may be a critical component in the reproductive biology of females.

Urinary constituents of some ungulates have been characterized. In red deer *(Cervus elaphus)*, urine consisted mainly of carboxylic acids and their derivatives, and some aromatic compounds (Bakke and Figenschou 1990). Volatiles identified in the urine of white-tailed deer *(Odocoileus virginianus)* belong to the alcohol, aldehyde, furan, ketone, nitrile, alkene, alkane, thiol ester, disulfide, aromatic, ether, ketal, and amine classes of compounds (Miller et al. 1998). No studies have characterized urinary compounds and putative pheromones of moose.

Some evidence exists that primer pheromones occur in the urine of ungulates (Watson and Radford 1960, Verme and Ozoga 1987). Synchronization of estrus and subsequent parturition would be of particular importance in ungulates because late-born young may experience higher rates of mortality (Clutton-Brock et al. 1984, Keech et al. in press). Additionally, timing parturition to coincide with resources necessary to meet the requirements of mother and young may be critical for successful reproduction (Bowyer 1991, Rachlow and Bowyer 1994, Bowyer et al. 1998a).

We tested the hypothesis that there were differences in the chemical composition of urine from adult male moose during rut compared with the same nonrutting adult males, a subadult male, or male and female young, and characterized those substances. These data are a necessary first step in understanding the potential role of pheromones in the reproductive biology of moose.

METHODS

Urine samples (≥200 ml) were collected from captive moose at the Kenai Moose Research Center, a facility operated by the Alaska Department of Fish and Game, on the Kenai Peninsula, Alaska, USA (60°N, 150°W). Urine was collected by holding a 1 liter-bottle affixed on a 1.8 m-pole while the animal urinated. Urine of 5 animals was collected in August and September 1991 by personnel of the Alaska Department of Fish and Game. Samples consisted of urine from rut, and from the nonrutting period for the same 2 adult males (≥8 years old), 1 yearling male (subadult), and 1 male and 1 female young (<1 year old) were sampled prior to rut in August. Samples were placed in a freezer at -20°C until analysis began.

Frozen samples were thawed in a coldroom at 4°C. The pH of each sample was determined before adjustment of pH and extraction. We obtained 3 fractions based on pH (acidic pH 1, neutral pH 7, and basic pH 12). Using the protocol of Menzies et al. (1992), we obtained organic compounds by 8 successive extractions with equal volumes of methylene chloride (CH_2Cl_2). We did not, however, perform extractions with $NaHCO_3$. The solvent was removed under reduced pressure by roto-evaporation at 40°C. The resultant extract was weighed and diluted with chloroform ($CHCl_3$) (100 μg/μl).

For preliminary analysis, we fractionated samples by thin-layer chromatography (TLC) on silica gel plates using a 2-solvent system gradient. Plates were developed first one-half way with a relatively strong eluting solvent, dried and then developed completely with a weaker eluting solvent. The basic fractions, however, were developed only with a single-solvent system (Table 1).

We analyzed volatiles and semi-volatiles on a Hewlett-Packard (HP) 5890 Series II gas chromatograph interfaced with a mass selective detector (HP 5972). We injected 1 μl onto a HP 5% phenyl methyl siloxane capillary column (0.25-μm x 30-m) using a flow rate of 1 ml/min, and an injection temperature of 275°C. The initial oven temperature was maintained at 50°C for 3 min and then increased to 300°C at 4°C/min. Peak identification was accomplished by matching mass spectra and retention times with authentic samples. For comparison of total number of peaks as detected by GC-MS, compounds with peaks >5 times the baseline or noise level were considered major peaks.

Volatiles and semi-volatiles were analyzed on a Hewlett-Packard (HP) 6890 Series II gas chromatograph equipped with a flame ionization detector. Samples of 1 μl were injected onto a HP 5% phenyl methyl siloxane capillary column (0.25-μm x 30-m) with a flow rate of 2.5 ml/min, and an injection temperature of 275°C. The initial oven temperature was maintained at 50°C for 3 min and then increased to 275°C at 5°C/min.

RESULTS

Urine collected in August (nonrut) from adult males lacked the characteristically pungent and musky odor of urine from rut. Additionally, qualitative differences were apparent in the urine of rutting and nonrutting adult moose as determined by thin-layer chromatography (TLC) on silica gel. A total of 11 TLC bands was unique to rutting males: 4 from the acidic fraction, 4 from the neutral fraction, and 3 from the basic fraction. Additionally, quantitative and qualitative differences in the chemistry of urine between rutting and nonrutting adult moose existed. In general, urine of nonrutting moose contained the greatest number of volatile and semi-volatile compounds (peaks) as detected by gas chromatography-mass spectrometry (GC-MS) (Figures 1, 2, and 3). The number of major chromatographic peaks in the acidic, neutral, and basic fractions of urine from 2 adult males declined from a mean (± SD) of 28.3±7.9 during nonrut to 19.5±5.2 during rut (Table 2). The male subadult (10.7±6.1) and two young moose (5.7±5.4) had fewer chromatographic peaks during nonrut than did adult males (Table 2).

Five urinary compounds were identified from the urine of moose. Dimethyl sulfone was common to all samples except those from rutting males. Butyrolactone was present only in the samples from adult males. Para-cresol occurred in the urine of all males. Although qualitative differences existed between the amount in rutting versus nonrutting urine, the amount of *p*-cresol in rutting samples was 5.5 times greater than for nonrutting males (Figure 1). Benzoic acid was present in samples of all males except for adult rutting males. Lupanine, which was tentatively identified using mass-spectra analysis, and sparteine only occurred in the sample from the female.

Two of 7 chromatographic peaks unique to rutting males were characterized by mass spectra analysis and are likely homologs of tetrahydro-6-methyl pyranone, and 2-nonen-4-one. Two additional peaks have been characterized as unsaturated alcohols.

DISCUSSION

Olfactory communication and associated scent-marking activities play a major role in the behavior and ecology of many species of mammals (Vandenberg 1983, Bowyer et al.

1998b). During the mating season, scent-marking behaviors associated with urine of male cervids has been demonstrated to be an important chemical cue to relay information to conspecifics (Bowyer and Kitchen 1987, Miquelle 1991). Specifically, adult male moose dig rutting pits in which they urinate, and females uniquely respond to the urine deposited in these pits. Although male yearling moose attempt to gain access to urine in the rutting pit, the chemical cues contained in adult urine are likely directed toward adult female moose. Indeed, primer pheromones may exist in the urine of some ungulates (Watson and Radford 1960, Schwartz et al. 1990). Because of the association and chronological order of events of moose courtship and copulation behavior, urine of rutting males likely is directed toward females and functions as a primer pheromone (Miquelle 1991).

Ungulates excrete large quantities of aromatic metabolites in their urine primarily because of the ingestion of cellulose plant materials. These metabolites are the excretory products of dietary phenolic acids, alicyclic acids, and aromatic amino acids that are first fermented by rumen microbes and then further metabolized in body tissues after absorption (Martin 1973, 1982a). The chemical composition of urine from adult male moose during rut is markedly different when compared with nonrutting adults (Figures 1, 2, and 3), a subadult male, male young, and female young. The urine of adult rutting males contained fewer volatile and semi-volatile peaks than from periods outside rut, which is in agreement with the findings of Martin (1970) that the quantity of various aromatic acid fractions excreted is directly proportional to the amount of food consumed. Typically, male moose become hypophagic and rely on the catabolism of endogenous reserves during rut to meet the metabolic needs of the animal (Miquelle 1990).

Bacterial interaction with exocrine secretions is responsible for the pungent axillary odor in humans (Leyden et al. 1981). Additionally, Stern and McClintock (1998) demonstrated for humans the importance of female axillary secretions (pheromones) and their associated bacterial fauna (Leyden et al. 1981) in the possible role in synchronization of the menstrual cycle. In moose, however, bacterial interaction does not appear to be important in the production of pungent urine during rut because the odoriferous urine of rutting males was freshly collected, and bioactivity was retained (i.e., females were attracted to the smell of the urine) (personal observation). Additionally, females respond immediately to males urinating in pits under natural conditions (Miquelle 1991); there would not be sufficient time for bacterial interactions in that process.

Four of 5 compounds identified in moose urine are common constitutes in the urine of many species. These compounds include butyrolactone, dimethyl sulfone, *p*-cresol, and benzoic acid (Figure 1). Sparteine appears to be unique to the urine of young female moose, but also has been reported in both genders of humans (Pfandl et al. 1992).

Butyrolactone occurred only in the urine of adult males. This compound also has been isolated from the sternal gland of the cockroach *(Nauphoeta cinerea)*. In the cockroach, however, this substance has not been identified as a pheromone (Sirugue et al. 1992). In rutting male moose, butyrolactone, may interact synergistically with other compounds in the urine to produce a chemosignal. Also, loss of a compound may alter odor and thereby act as a chemical signal. For example, dimethyl sulfone was present in the urine of nonrutting males but did not occur in rutting adults (Figure 1).

P-cresol was identified only from the urine of males. A substantial difference occurred in the quantity of *p*-cresol in rutting versus nonrutting males (Figure 1). *P*-cresol is an end-product of anaerobic microbial degradation of tyrosine (Bakke 1968, Bone et al. 1976, Spoelstra 1978). Therefore, the quantitative differences of *p*-cresol may result from the breakdown of endogenous reserves during rut when moose are hypophagic for about 18 days (Miquelle 1990). That outcome, however, does not preclude its use as a pheromone.

P-cresol has been identified as a component of the pheromone in 2 species of hard tick *(Rhipicephalus appendicalatus* and *R. pulchellus)* (Wood et al. 1975) and cabbage looper *(Trichoplusia ni)* (Heath et al. 1992). An insect pheromone has functioned as a mammalian pheromone; Rasmussen et al. (1997) demonstrated that a lepidopteran pheromone occurred in the urine of female Asian elephants *(Elephas maximus)*, and that compound was identified as a female-to-male sex pheromone. Perhaps *p*-cresol serves a similar function in moose.

Benzoic acid occurred only in the urine of nonrutting males. This trend is in agreement with studies investigating the role of malnutrition on the excretion of urinary compounds; the concentration of benzoic acid in the urine is correlated positively with the amount of food eaten (Martin 1969, Martin 1970, Martin, 1973, Silanikove and Brosh 1989). This concept holds for adult male moose that undergo hypophagia during rut (Miquelle 1990).

Sparteine and lupanine occurred only in the urine of female young. These naturally occurring quinolizidine alkaloids are present in leguminous plants including the genus *Lupinus*. Although the moose in this study were given a commercial pelleted diet, there is an opportunity for them to feed on indigenous vegetation, including species of *Lupinus*.

Two of 7 chromatographic peaks unique to rutting males were tentatively identified by mass-spectra analysis and are most likely homologs of tetrahydro-6-methyl pyranone, and 2-nonen-4-one Two additional peaks have been characterized as unsaturated alcohols. That these compounds are unique to rutting males may indicate that they are involved in pheromonal activity.

Airborne pheromones usually contain between 5 and 20 carbon atoms and must be volatile to reach the receiver. As such, they are constrained by their structure and the limits imposed by functional groups, and have molecular weights near 300 (Bradbury and Vehrencamp 1998). In white-tailed deer, several volatile compounds occurred exclusively in the urine of dominant males during the mating season that had molecular weights <300 (Miller et al. 1998). Similarly, in red deer *(Cervus elaphus)* volatile compounds had molecular weights <300 (Bakke and Figenschou 1990). Female Asian elephants *(Elephas* *maximus)* release a female-to-male urinary pheromone with 13-carbons and a molecular weight near 300 (Rasmussen et al. 1997). In moose, the urinary compounds that were unique to rutting males had molecular weights of <300 and had fewer than 20 carbon atoms, which meets criteria necessary to be an airborne pheromone.

Synchronization of estrus has been demonstrated in humans (Stern and McClintock 1998) and rats (McClintock 1984). In cervids, male pheromones likely prime and synchronize estrus (Coblentz 1976). Synchronization of estrus would be of particular importance in a cervid such as moose because timing parturition to coincide with resources necessary to meet the requirements of mother and young is critical for successful reproduction (Bowyer et al. 1998a, Keech et al. in press).

Digging of rutting pits and deposition of pheromones in the pit should be considered a secondary sexual character related to mating (Möller 1996). Expression of such sexual characters often shows evidence of being dependent on body condition (Andersson 1986). For example, cervids usually grow larger antlers as a direct response to individual physical condition (Van Ballenberghe 1982, Goss 1983, Stewart et al. in press). Such high-quality individuals can afford to invest relatively more in cost-reducing traits than can low-quality individuals (Zahavi 1975, Zahavi and Zahavi 1997). Rutting activities are costly for adult male moose. Not only do they engage in vigorous fights with other males but also reduce their intake of food, and rely mainly on endogenous reserves (Miquelle 1990). During rut, adult males can lose from 12 to 19% of their pre-rut body weight (Franzmann et al. 1978, Schwartz et al. 1987). The catabolism of endogenous reserves as a result of decreased food intake coupled with changes in androgens probably account for the pungent smell of urine during rut. Additionally, rut occurs at the time of year when Alaskan moose will be stressed physiologically by the subsequent demands associated with severe winters (Franzmann and Schwartz 1998). Only adult males incur the high energetic costs associated with rut. This cost-handicap may be a self-imposed test upon the male. A male with a well-developed sexually selected character is an individual that has survived that test (Zahavi 1975). A female moose that could discriminate a male of high quality can be assured that it has selected from among the best phenotypes, and presumably genotypes, and that this trait will be passed on to her male offspring (Zahavi and Zahavi 1997). Urine excreted during rut has a very characteristic smell. Sexually mature but young males seldom have strong odoriferous urine or dig rutting pits. We suggest that small males, which do not dig pits or produce pungent urine, attempt to cheat by impregnating their pelage by wallowing in the pits of larger males and acquiring a primer pheromone that is attractive to females.

Darwin (1859) proposed that the features and qualities of each species are formed by the process of natural selection, in which the more efficient survived and reproduced. In moose, successful males spent more time interacting with females and engaging in antagonistic behaviors with other males (Miquelle and Van Ballenberghe 1985). This increased activity lessened the amount of time for feeding. The most successful individuals were able to sustain such activities over long periods and ultimately relied on endogenous body reserves to do so. Catabolism of those reserves resulted in a suite of urinary metabolites that could be detected by conspecifics and provided an honest indication of body condition, supporting our hypothesis that those substances provide ideal candidates for pheromones in moose.

More studies need to be conducted to demonstrate whether the urine of adult male moose deposited in rutting pits contains primer pheromones. The chemical composition of urine of adult males during rut is markedly different when compared with nonrutting adult males, a yearling male, and male and female young. Adult male moose forage little during rut; therefore, some of the unique compounds in the urine at this time may be metabolic by-products of fasting. Because of the behavioral response of both females and subadult males to the urine of adult rutting males, we conclude that the unique compounds alone or synergistically may function as pheromones. To determine if compounds that are unique to urine of rutting males are pheromones, future studies should investigate the behavioral responses of females to male urine, and then specifically focus on the unique compounds and combinations of them to elucidate their role as primer pheromones.

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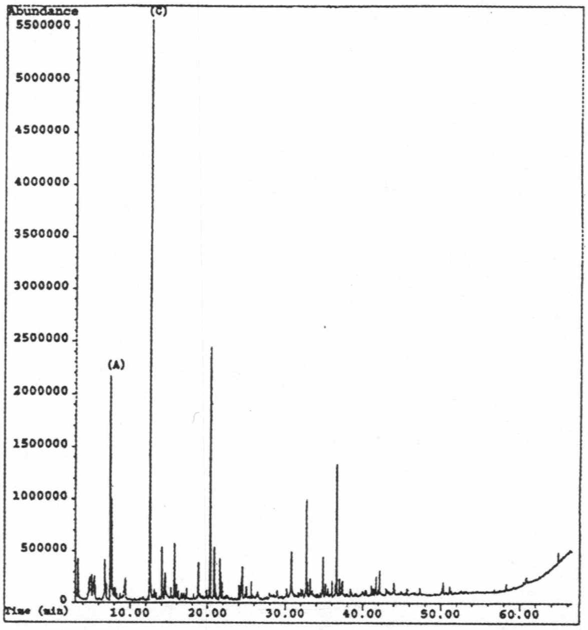
RUT



NONRUT

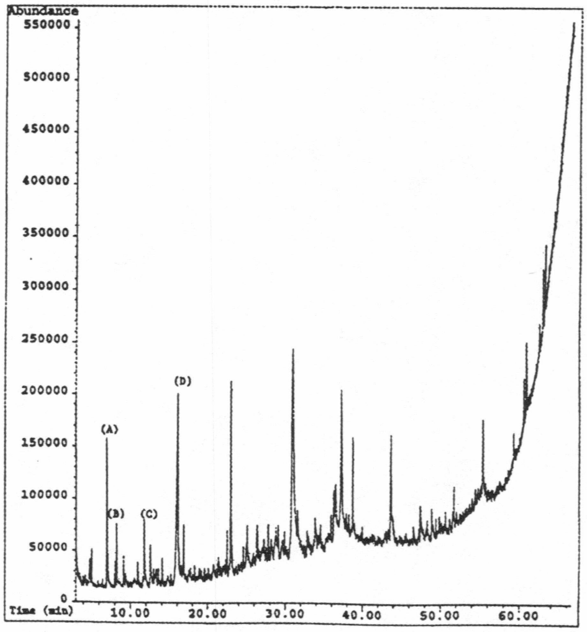
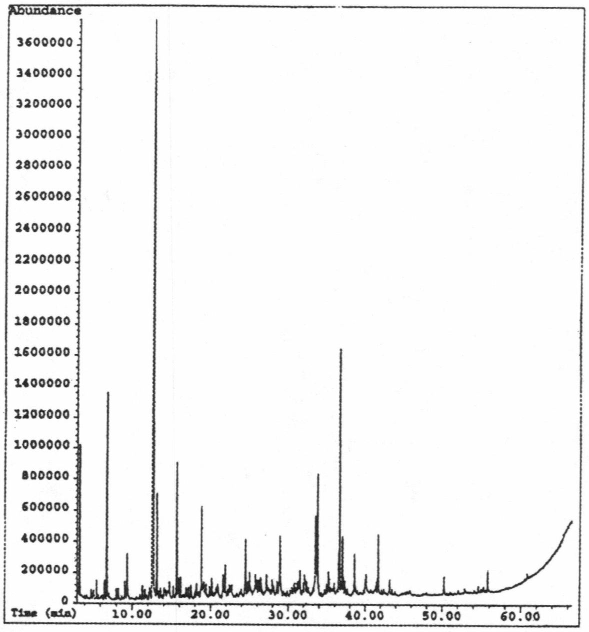


Figure 1: Chromatograph of urine from adult male moose during rut, and nonrut (acidic fraction): A) butryolactone, B) dimethyl sulfone, C) para-cresol, D) benzoic acid

RUT



NONRUT

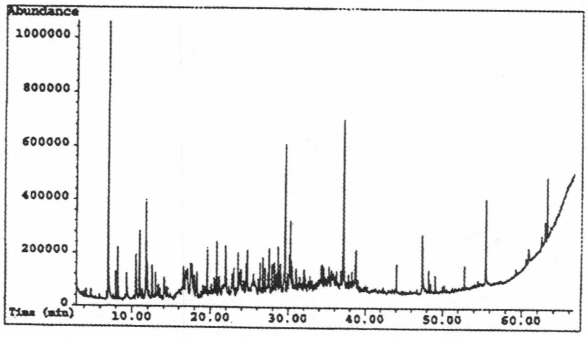
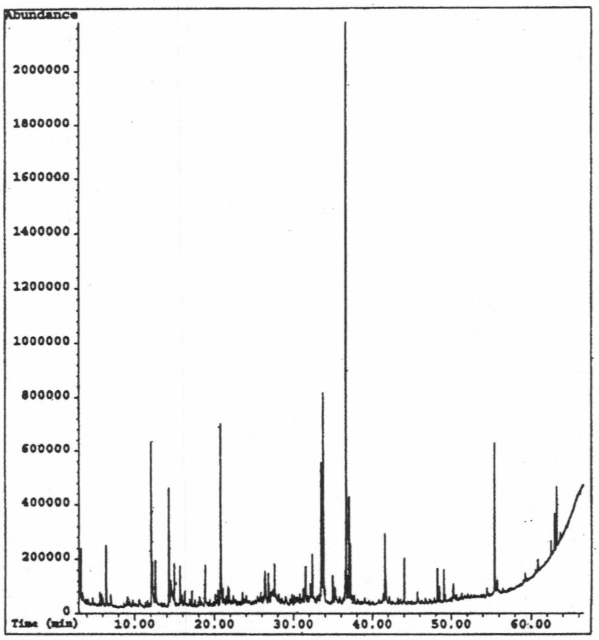


Figure 2: Chromatograph of urine from adult male moose during rut, and nonrut (neutral fraction)

RUT



NONRUT

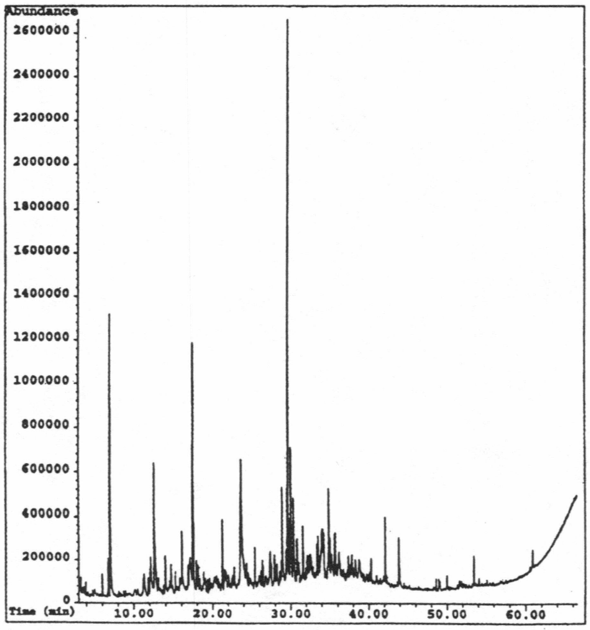


Figure 3: Chromatograph of urine from adult male moose during rut, and nonrut (basic fraction)

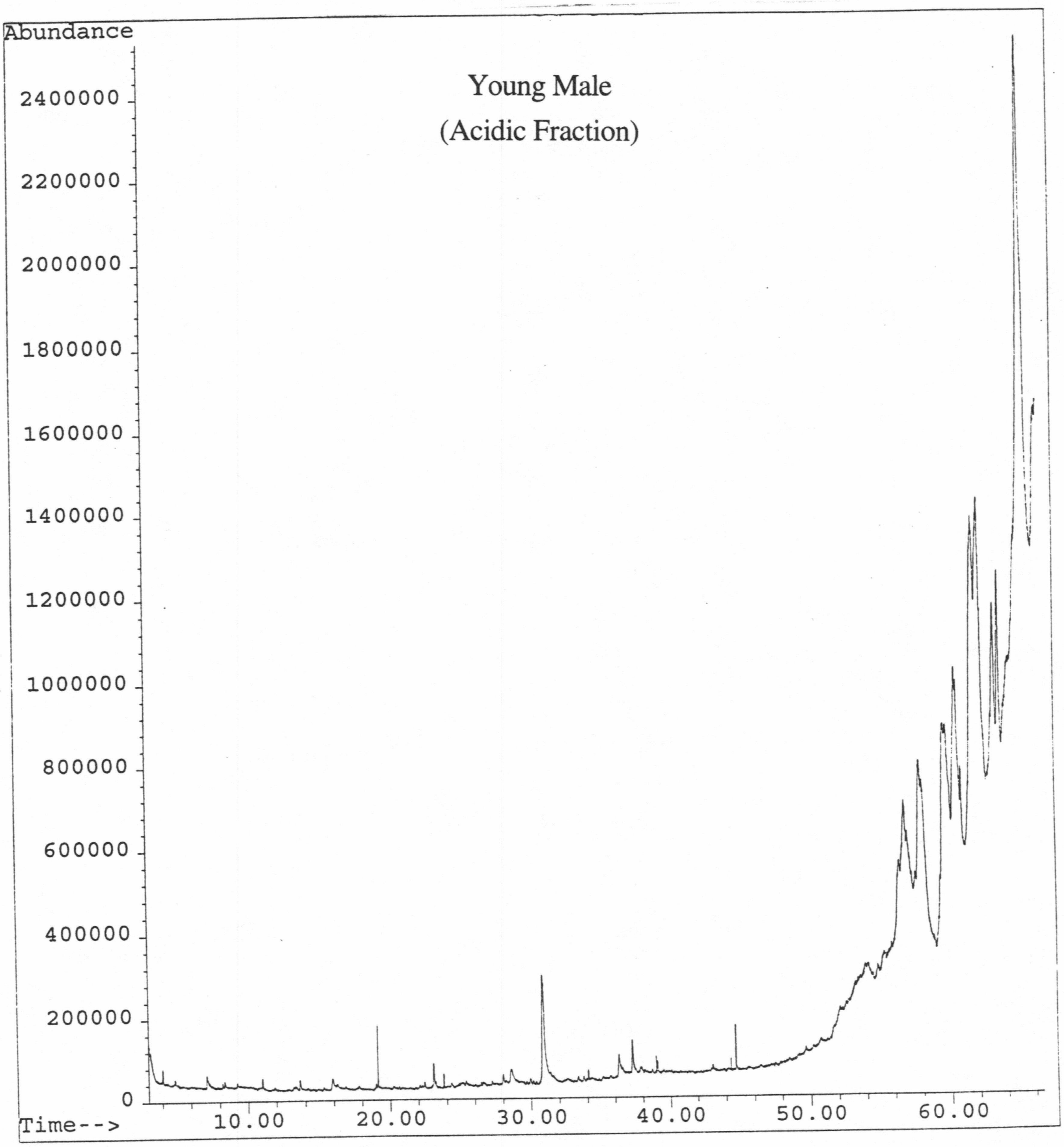
Table 1: Thin-layer chromatography solvent systems used to analyze extracted moose urine. Plates were developed first one-half way with a relatively strong eluting solvent (Solvent #1), dried and then developed completely with a weaker eluting solvent (Solvent #2).

Fraction	**Solvent #1**	**Solvent #2**
Acidic	$CHCl_3$:MeOH:H_2O (50:20:1)	$CHCl_3$:MeOH:H_2O (90:20:1)
Neutral	$CHCl_3$:MeOH:H_2O (90:20:1)	$CHCl_3$
Basic	$CHCl_3$:MeOH:H_2O (40:10:1)	

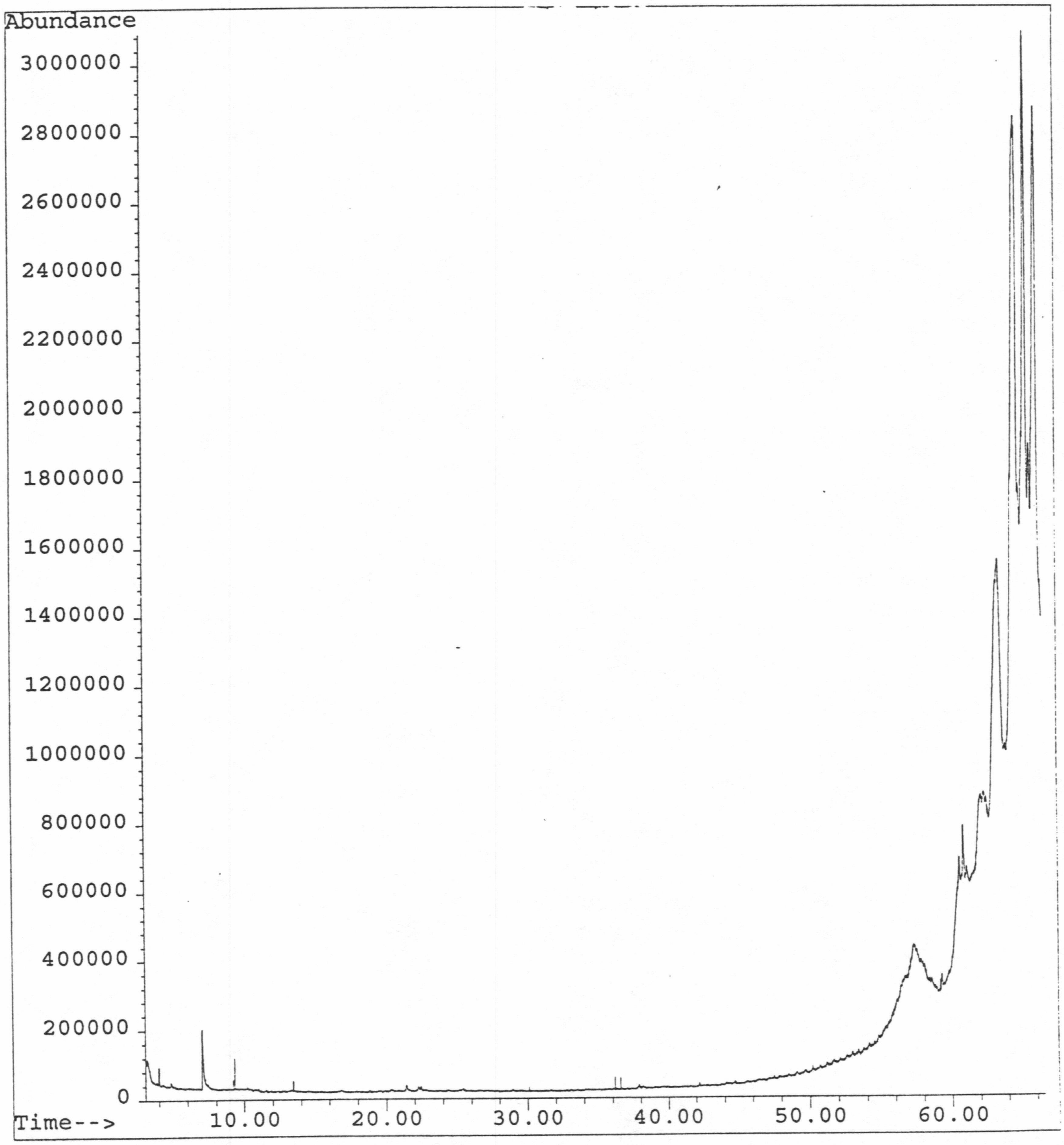
Table 2: Mean number of major chromatographic peaks of moose urine as detected by gas-chromatography-mass spectrometry for all classes of animals

Animal	Mean Chromatographic Peaks	S.D.
Adult males (nonrut)	28.3	7.9
Adult males (rut)	19.5	5.2
Subadult male	10.7	6.1
Male and female young	5.7	5.4

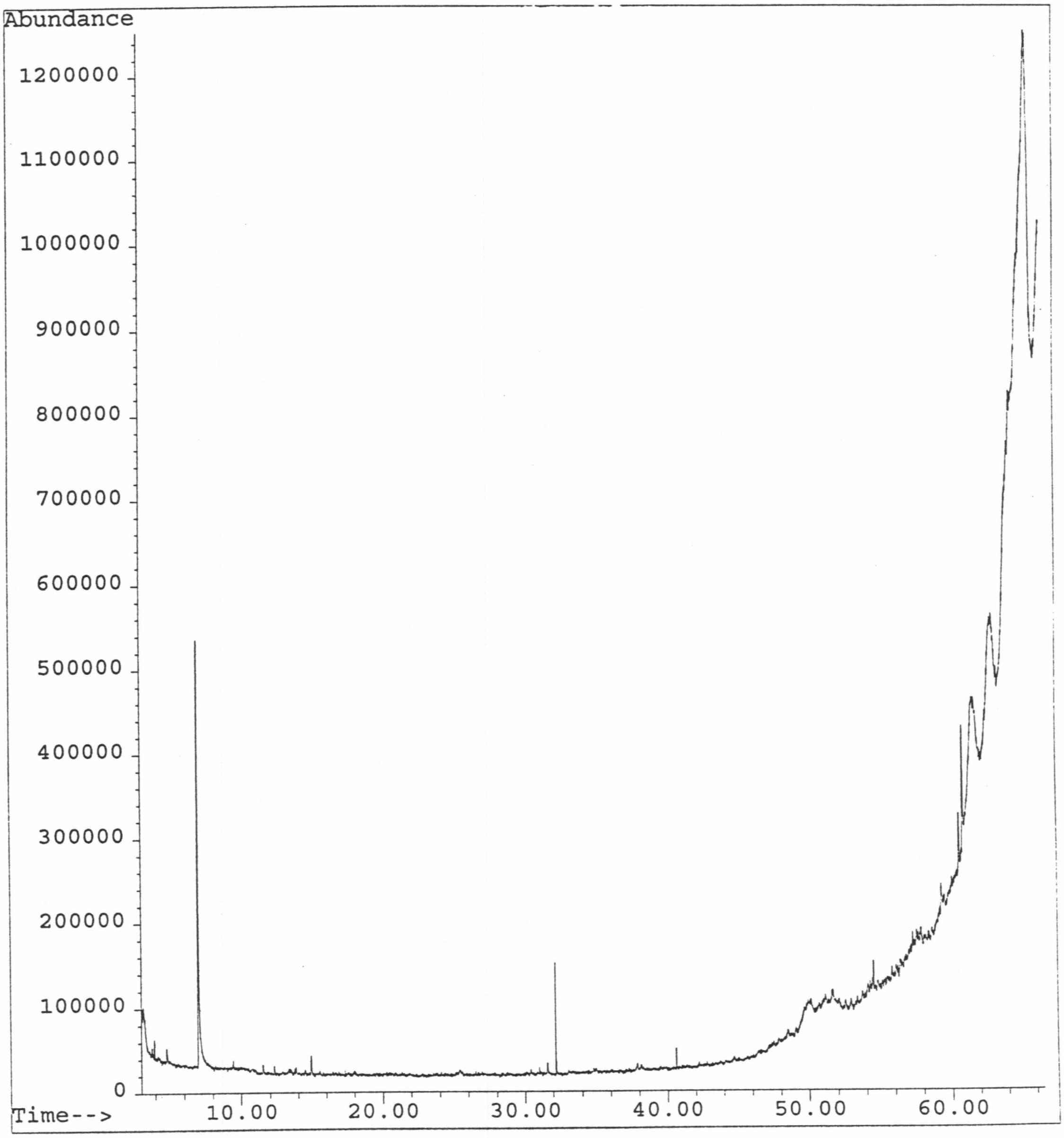
APPENDIX A
GAS CHROMATOGRAPHY-MASS SPECTROMETRY CHROMATOGRAPHS



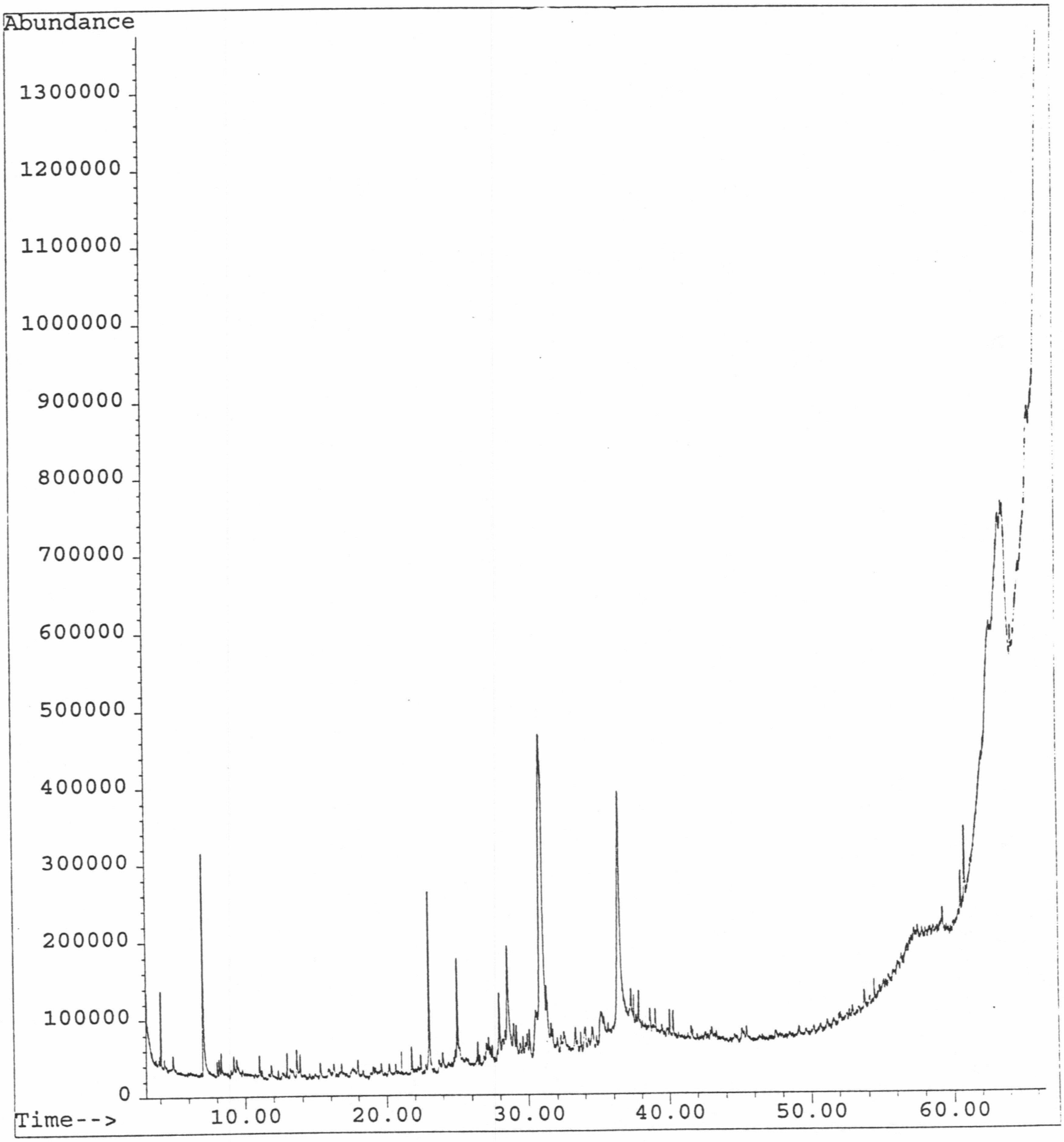
Young Male
(Neutral Fraction)



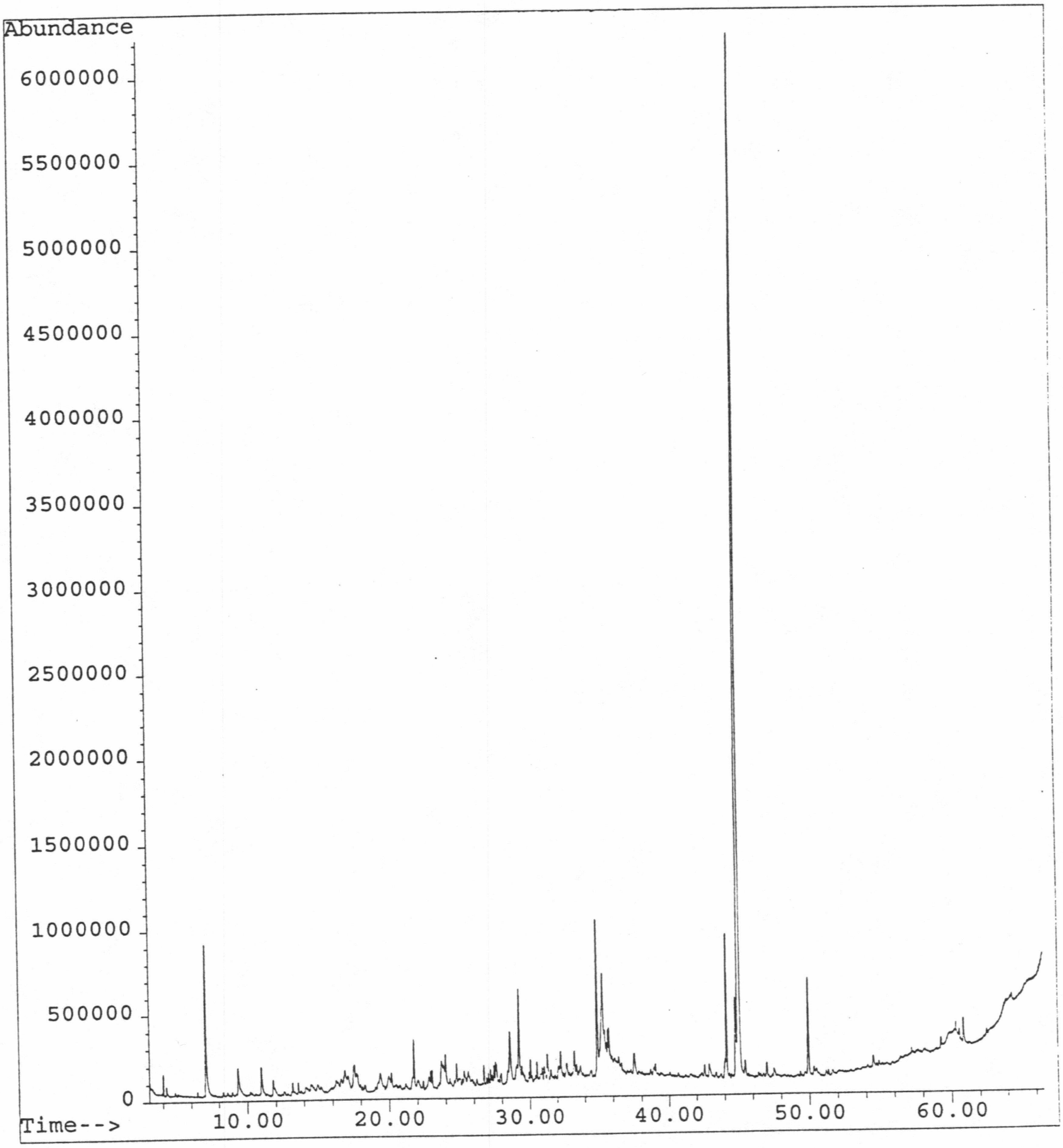
Young Male
(Basic Fraction)



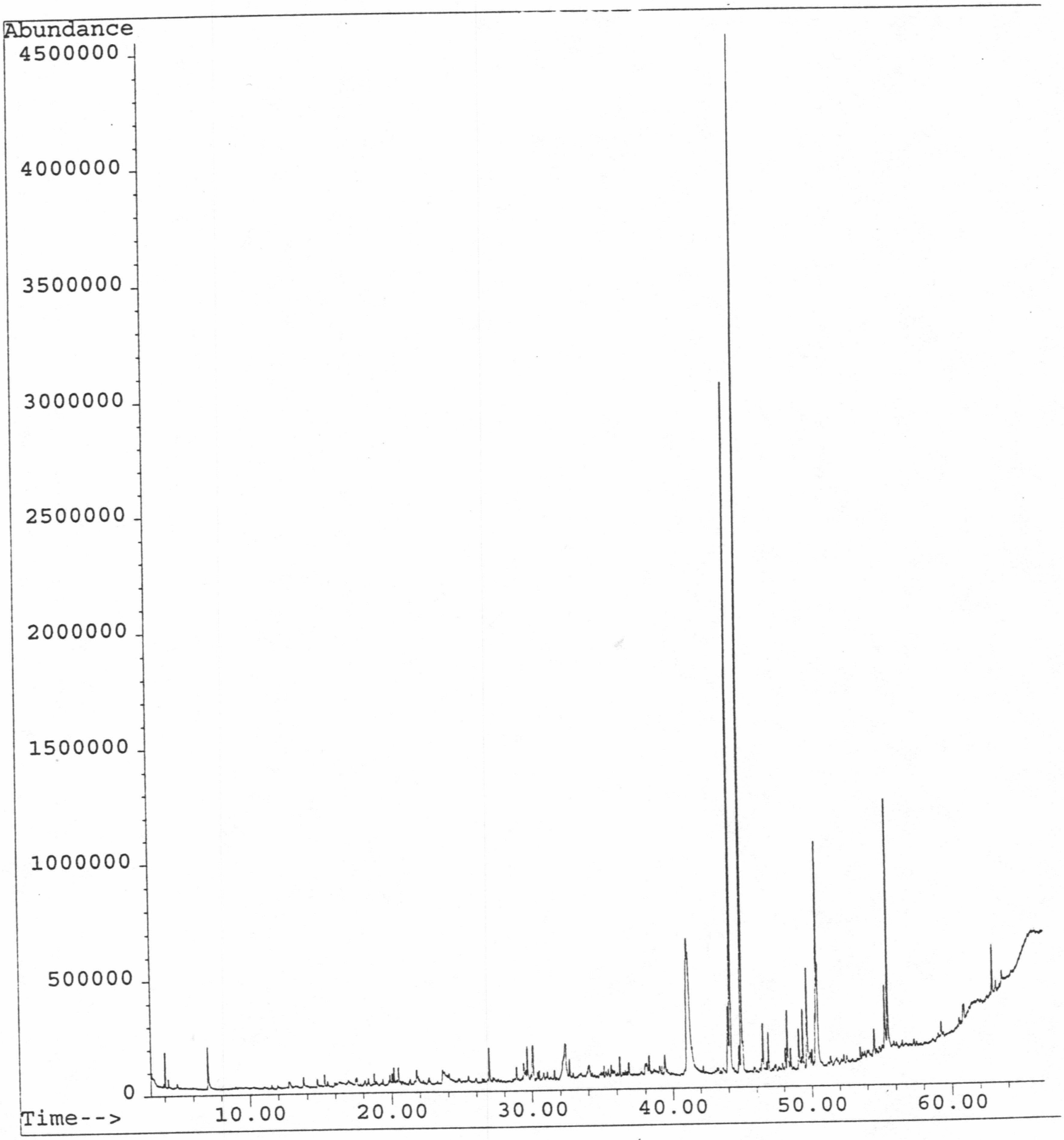
Female Young
(Acidic Fraction)



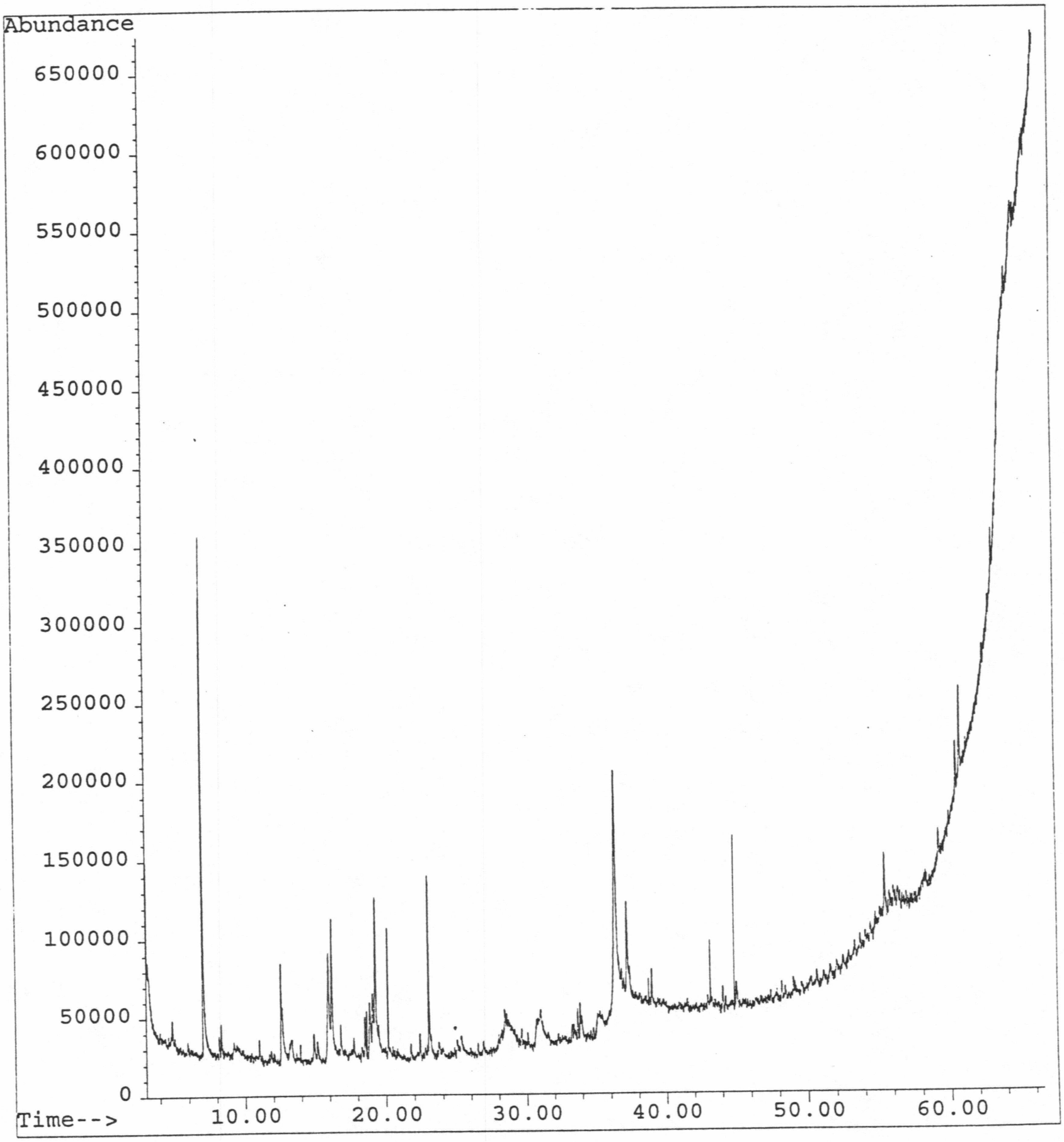
Female Young
(Neutral Fraction)



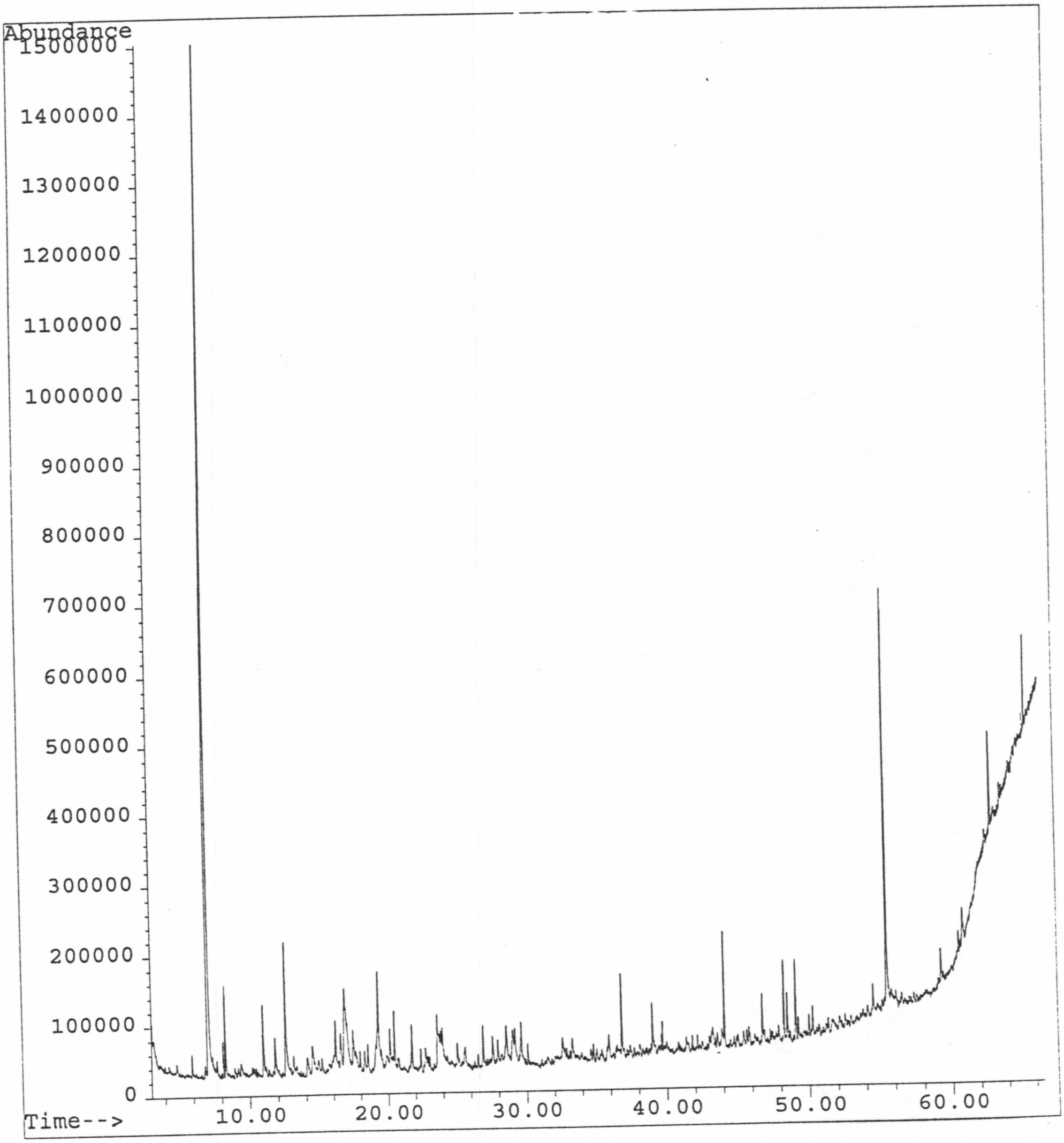
Female Young
(Basic Fraction)



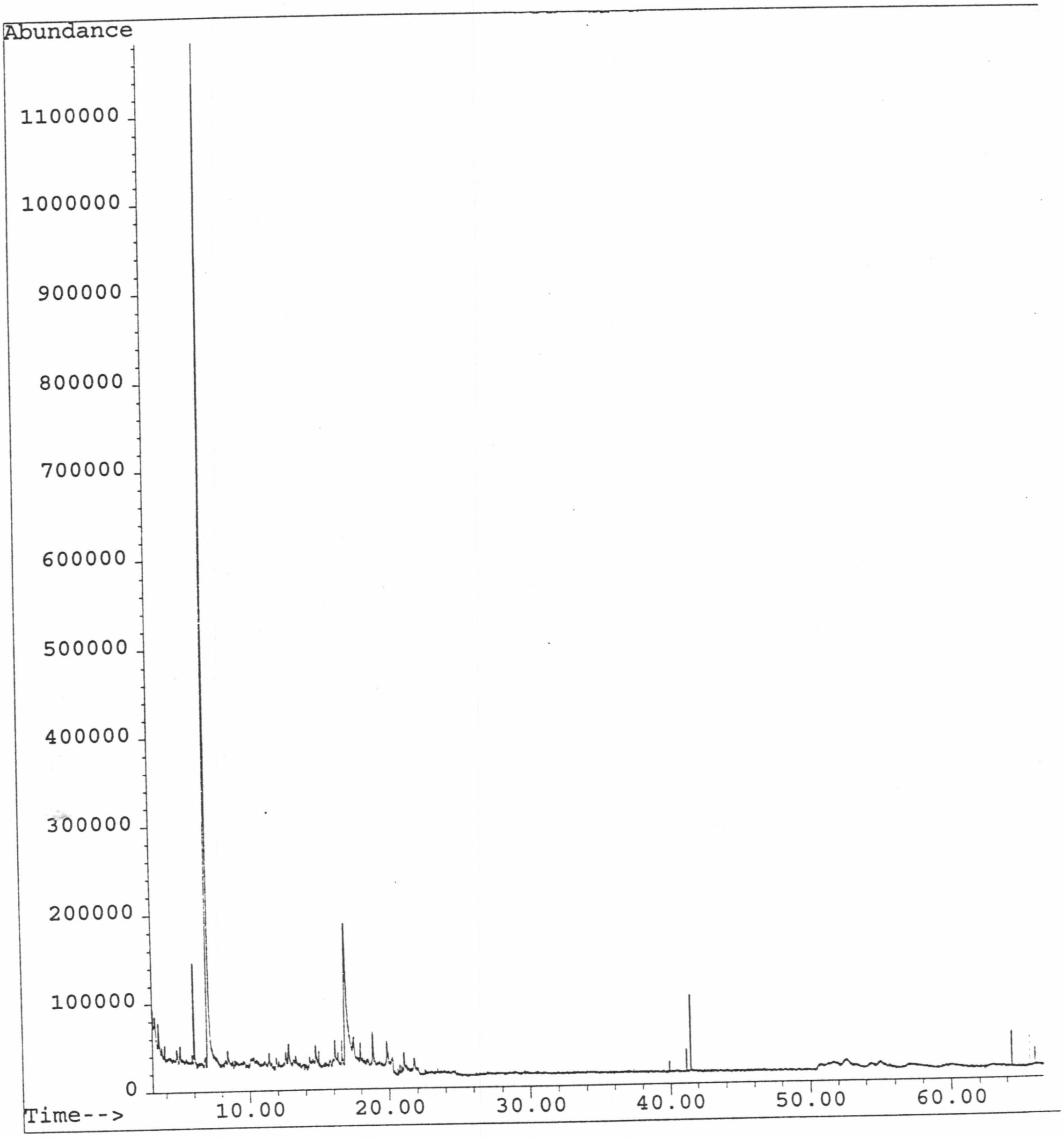
Subadult Male
(Acidic Fraction)



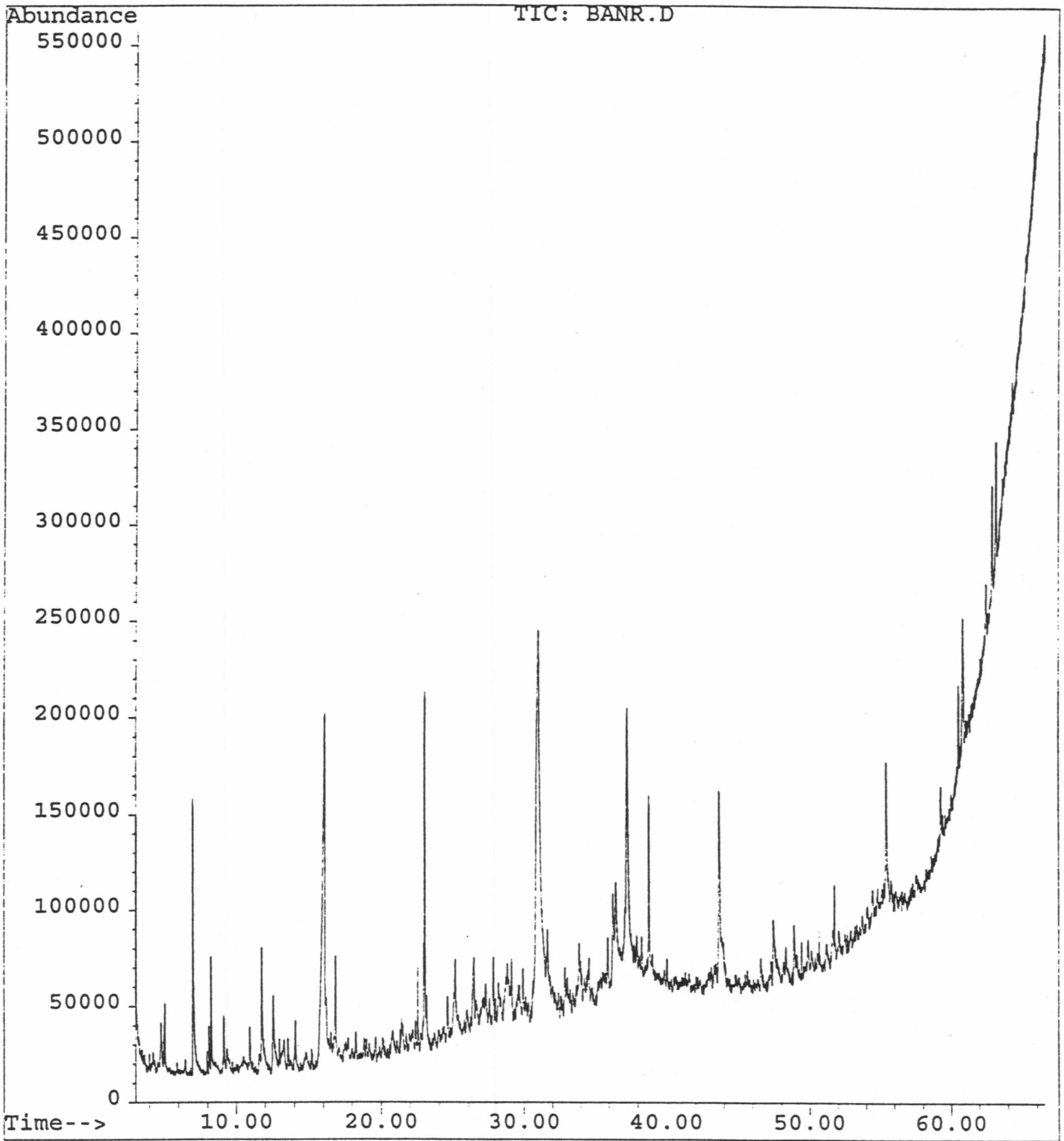
Subadult Male
(Neutral Fraction)



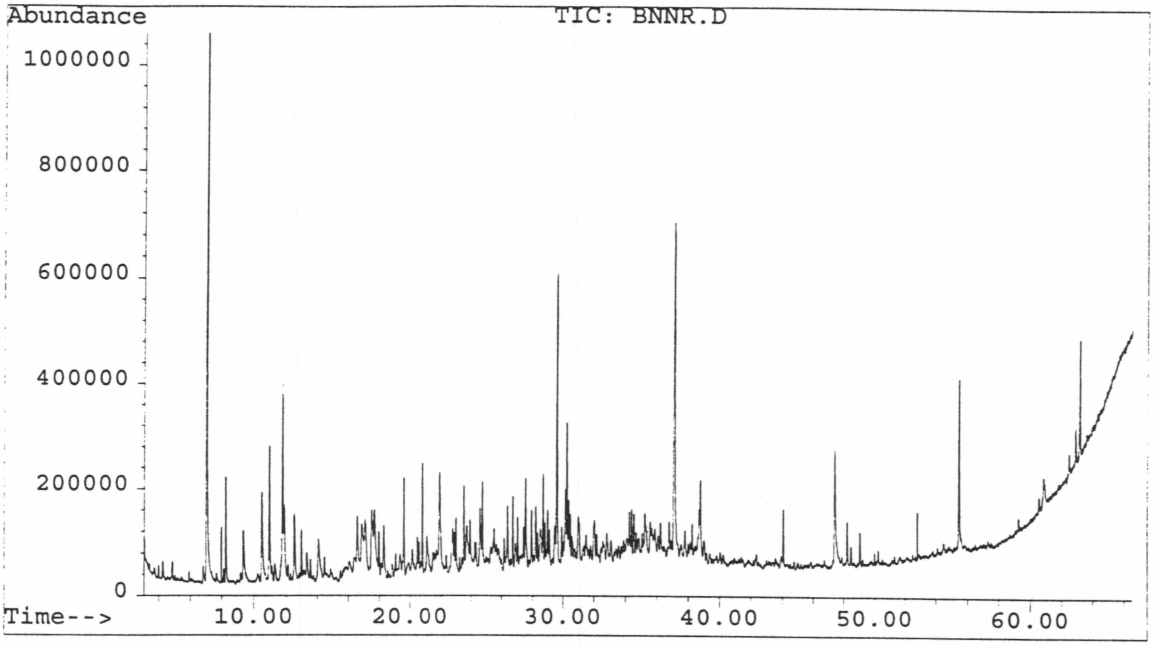
Subadult Male
(Basic Fraction)



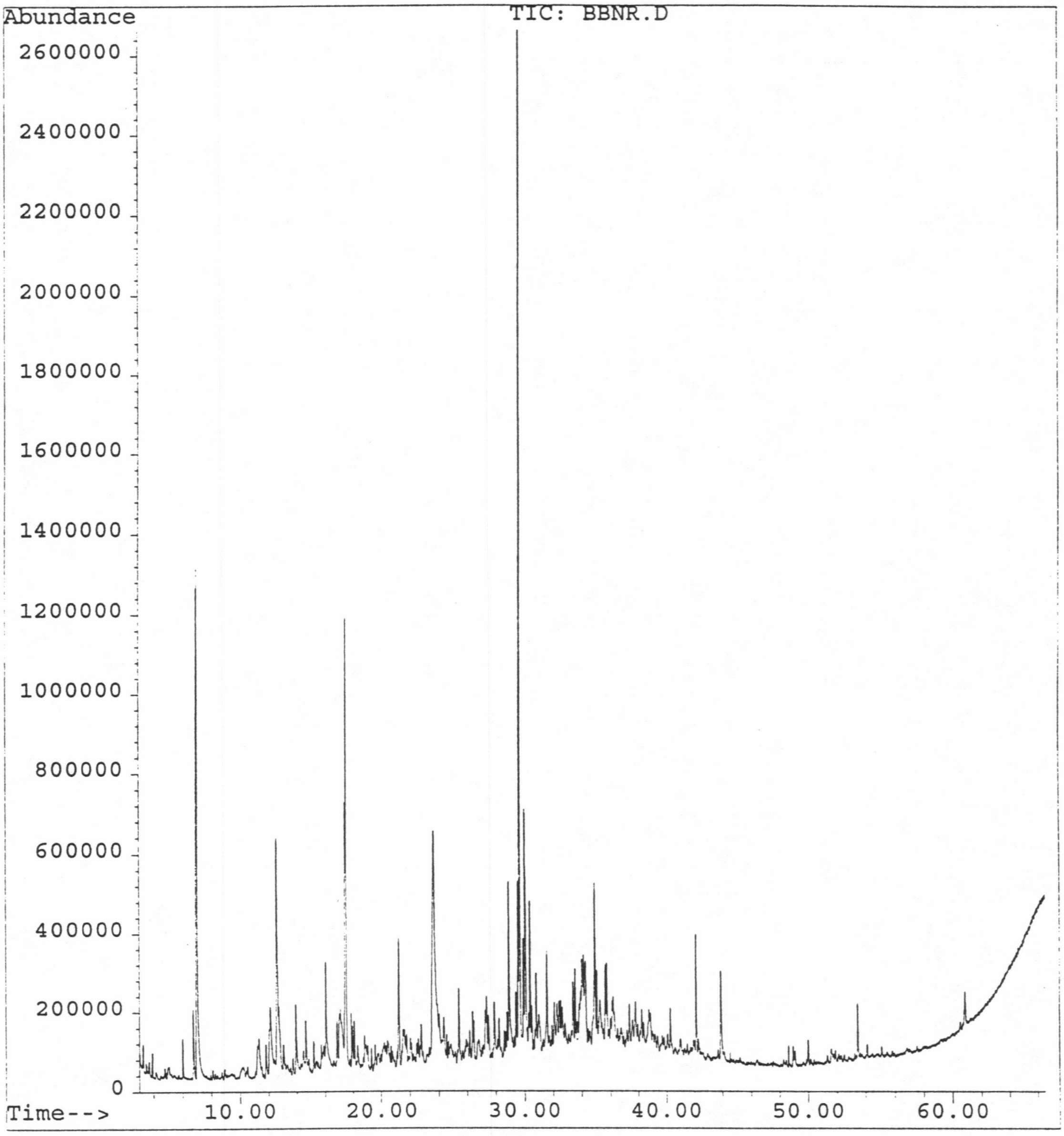
Adult Male #1 - Nonrut
(Acidic Fraction)



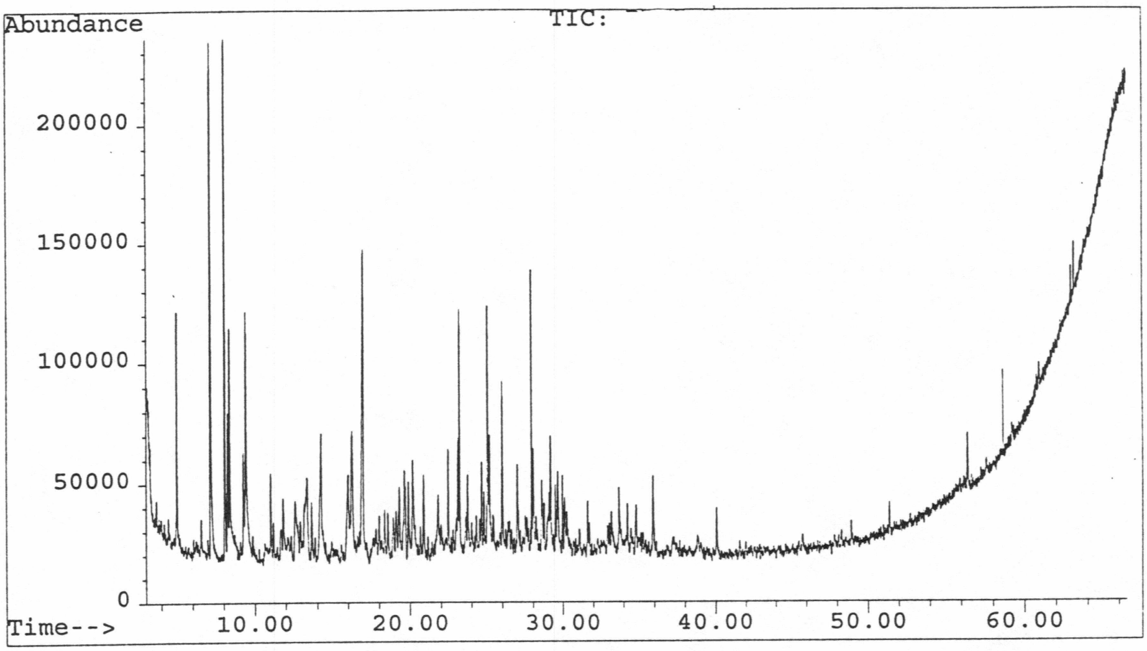
Adult Male #1 - Nonrut
(Neutral Fraction)



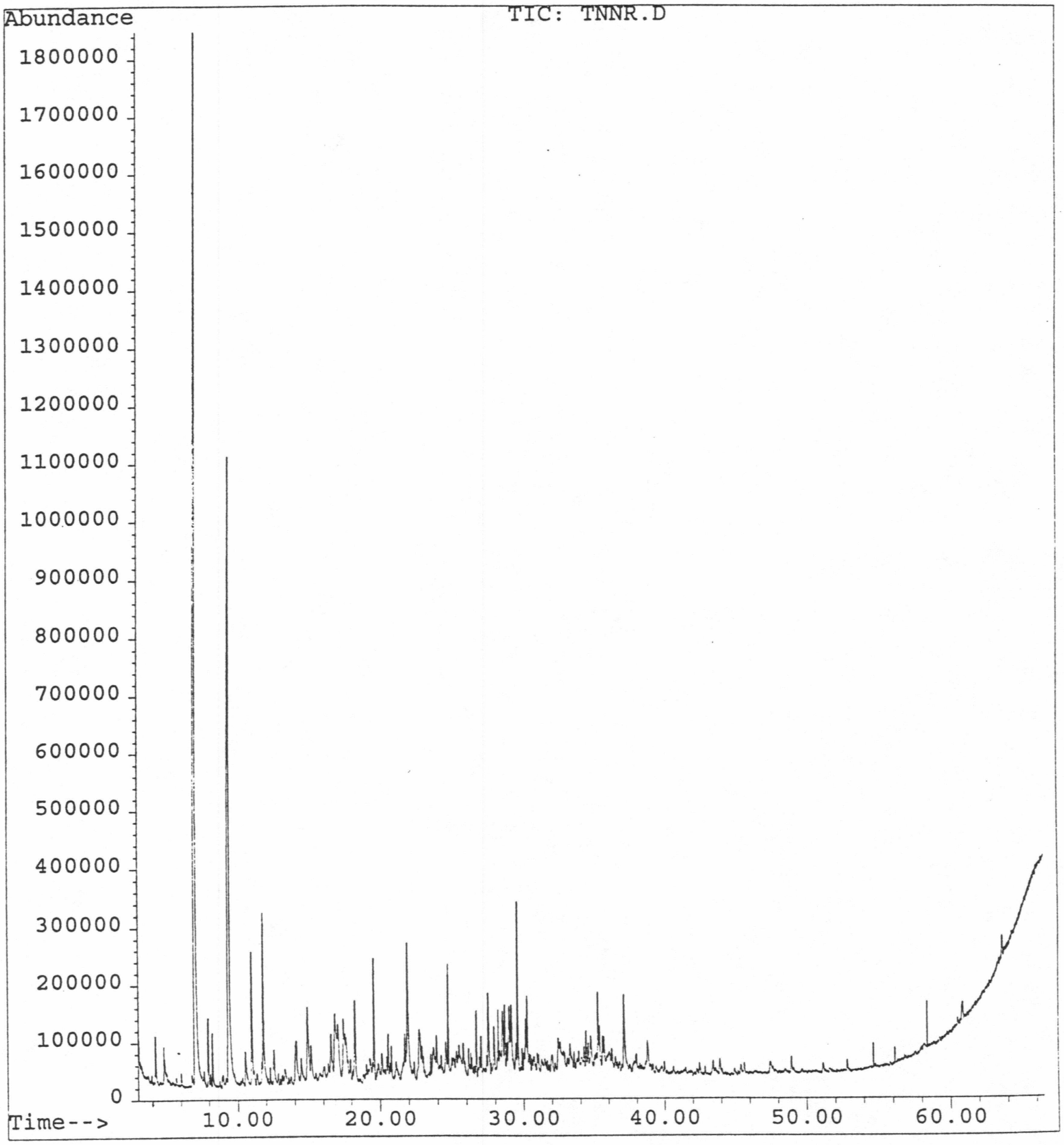
Adult Male #1 - Nonrut
(Basic Fraction)



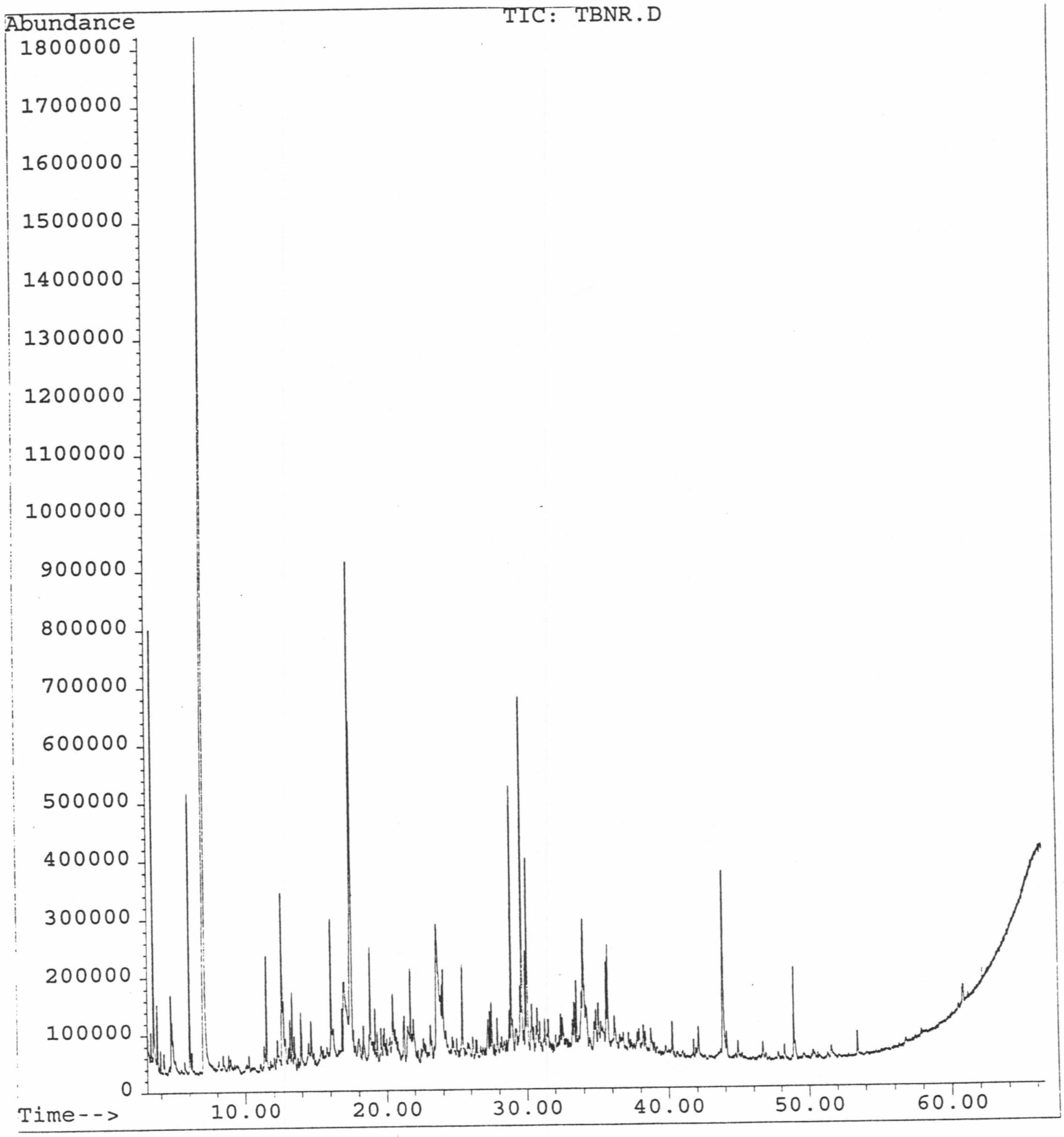
Adult Male #2 - Nonrut
(Acidic Fraction)



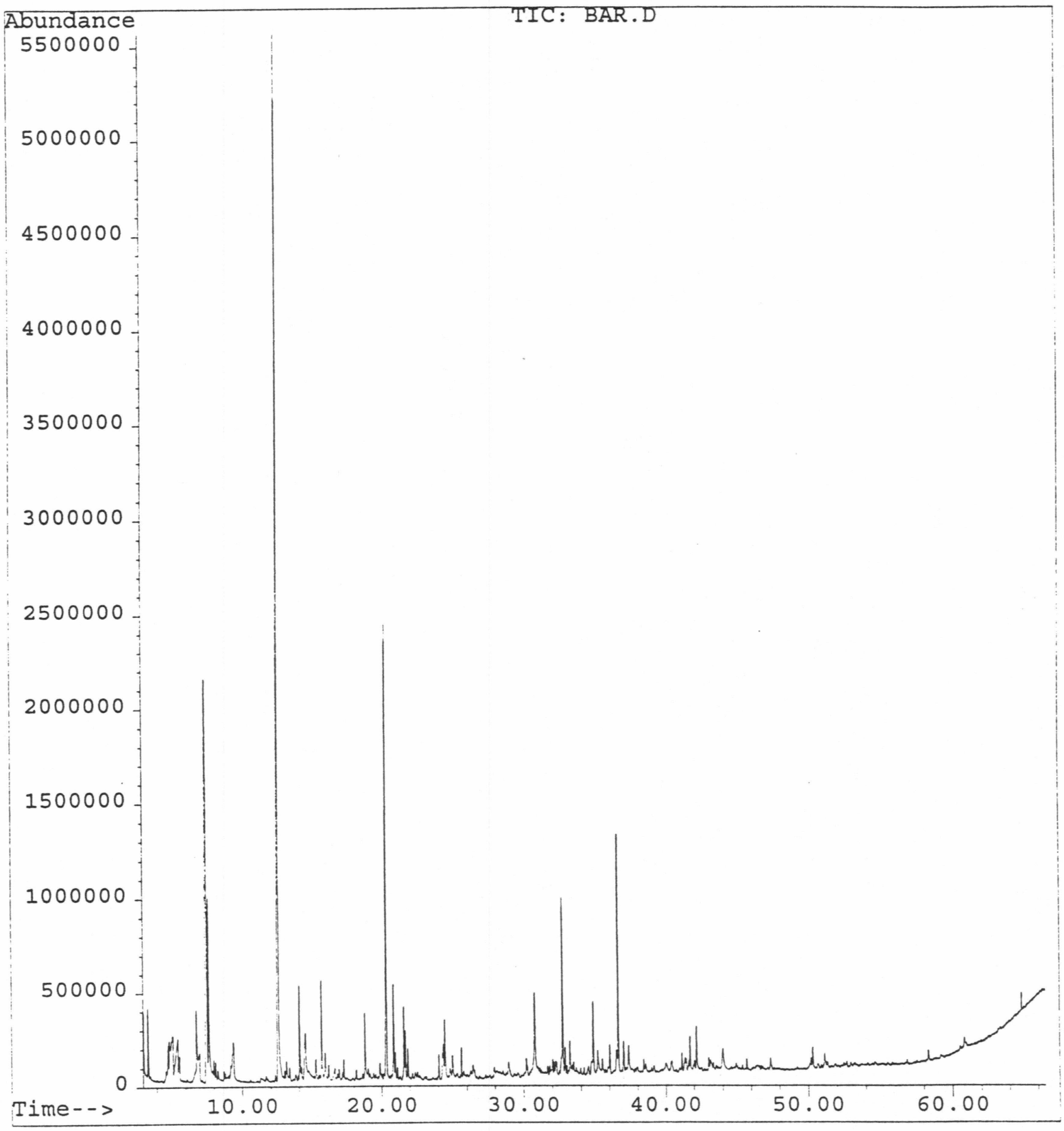
Adult Male #2 - Nonrut
(Neutral Fraction)



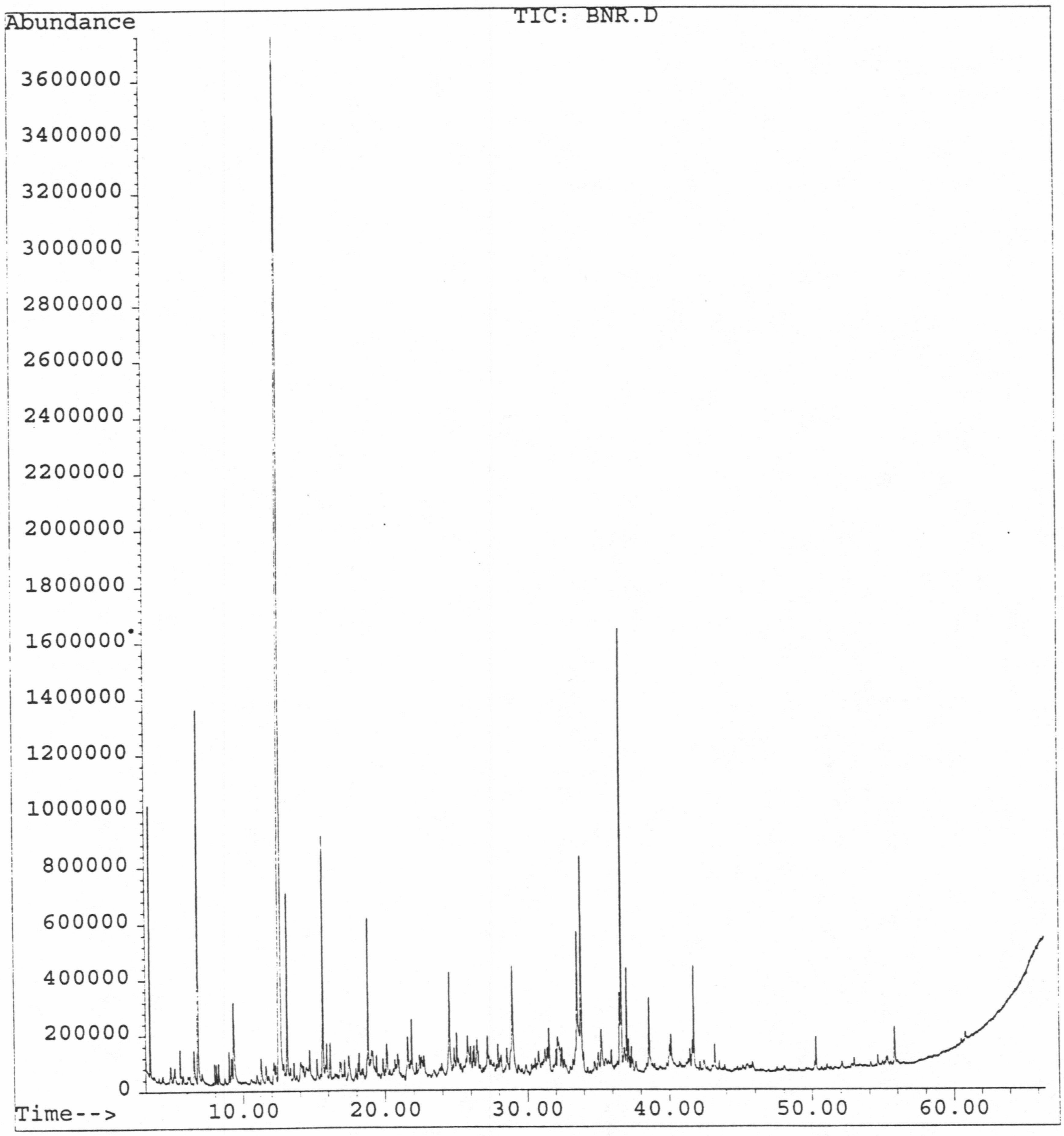
Adult Male #2 - Nonrut
(Basic Fraction)



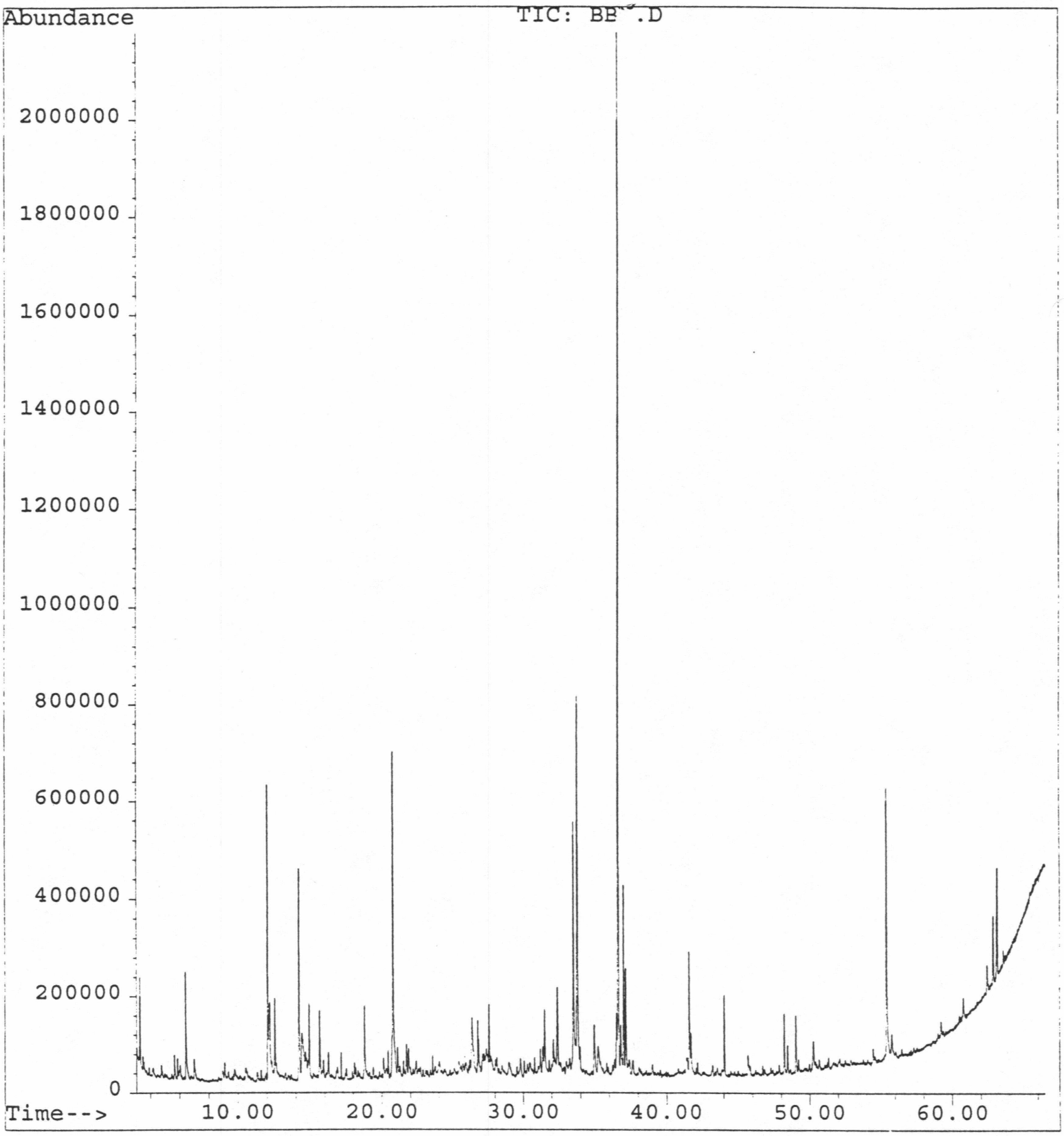
Adult Male #1 - Rut
(Acidic Fraction)



Adult Male #1 - Rut
(Neutral Fraction)

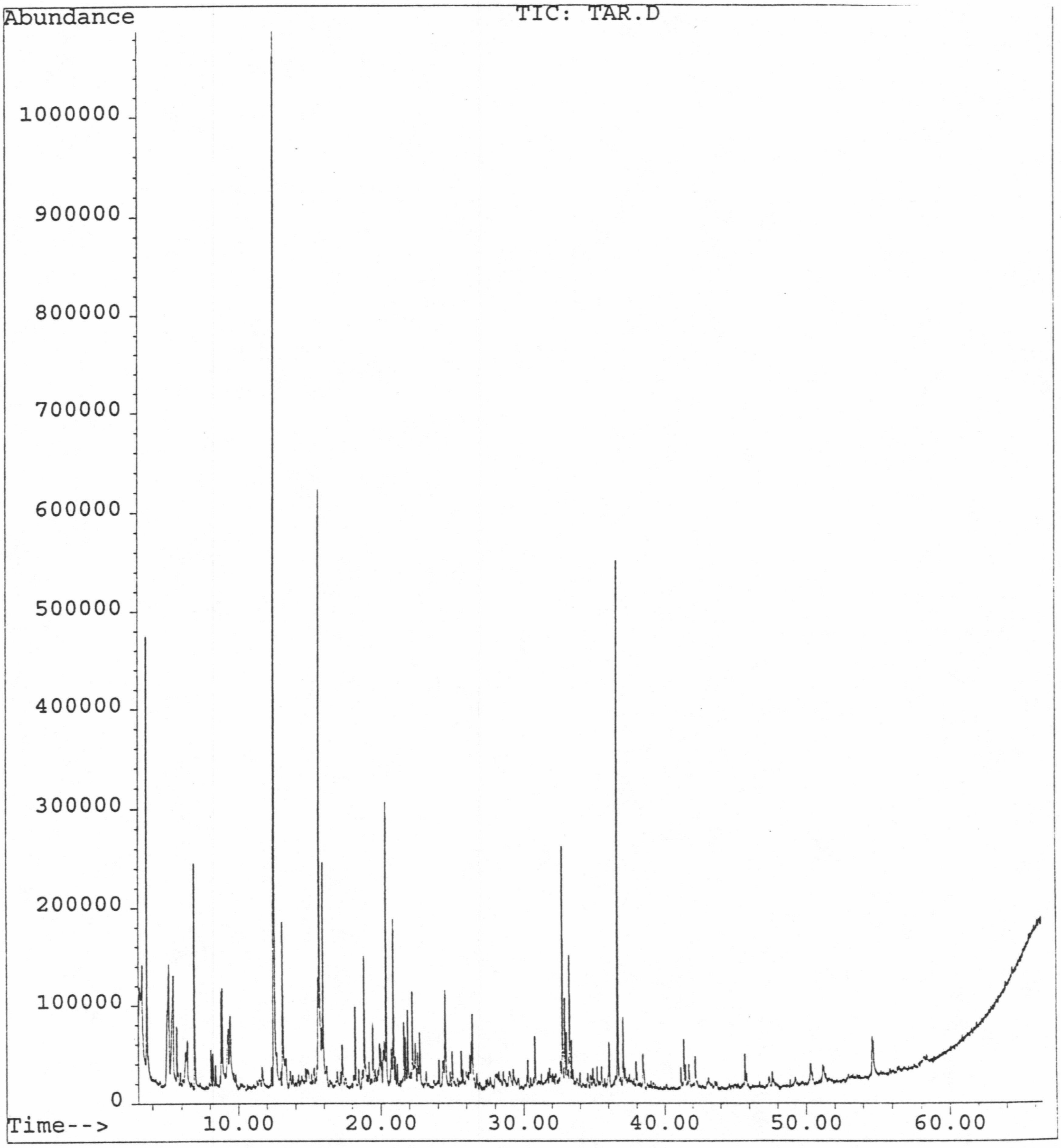


Adult Male #1 - Rut
(Basic Fraction)

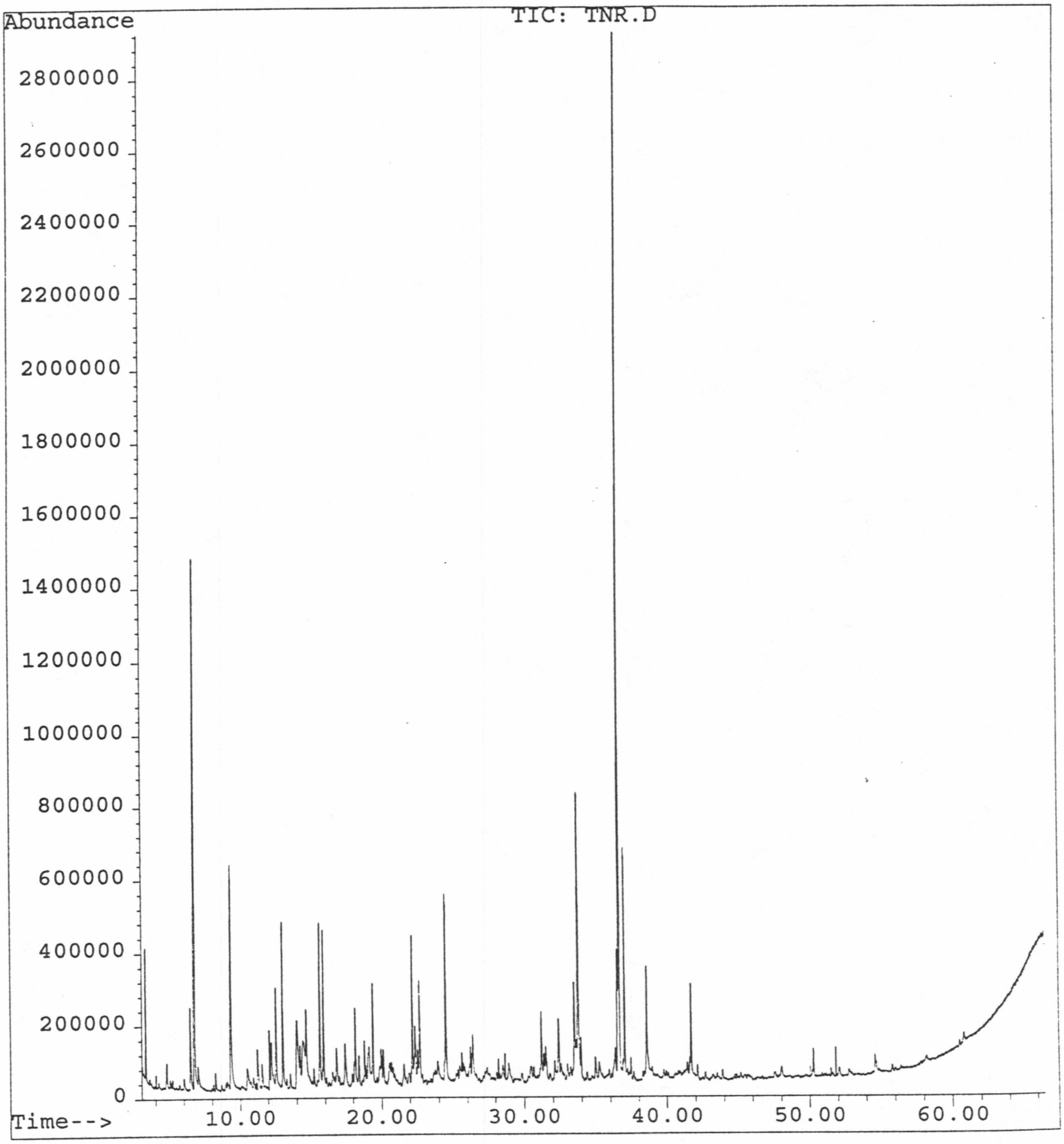


Adult Male #2 - Rut

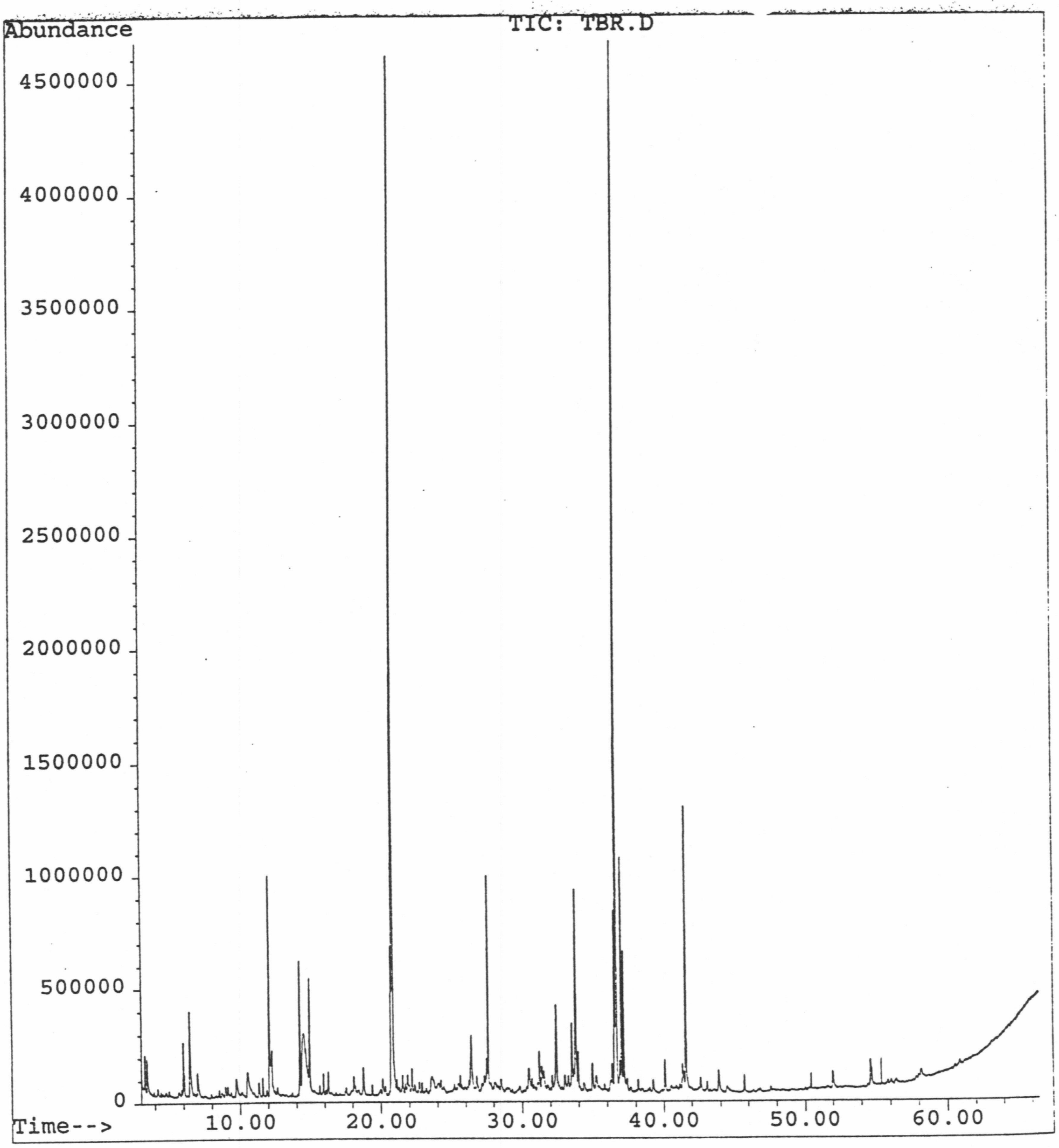
(Acidic Fraction)



Adult Male #2 - Rut
(Neutral Fraction)



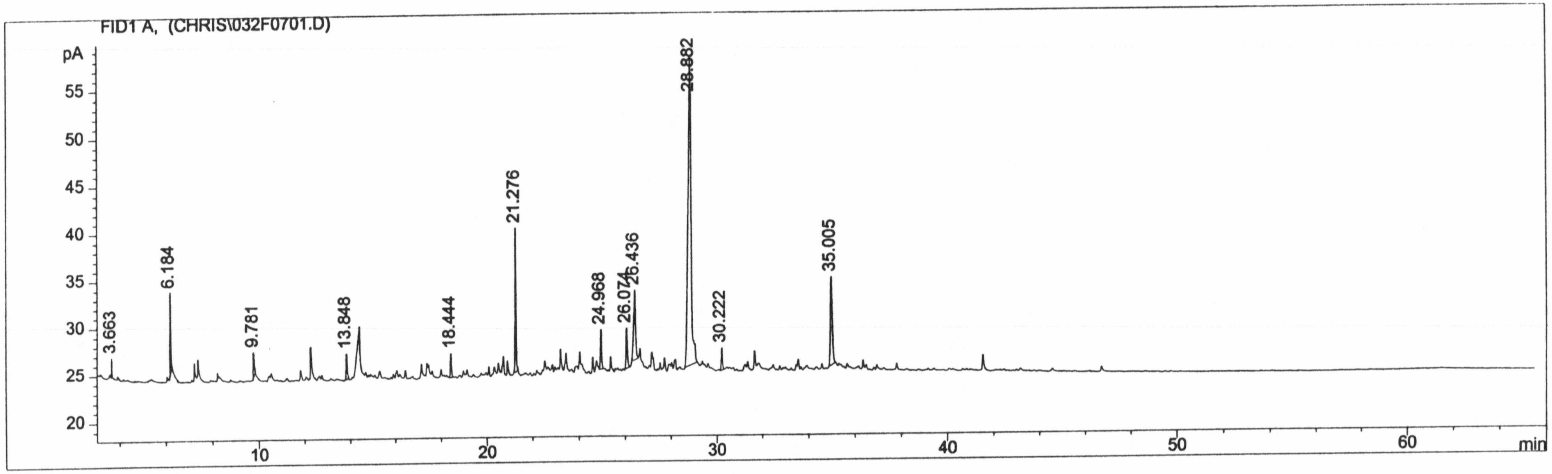
Adult Male #2 - Rut
(Basic Fraction)



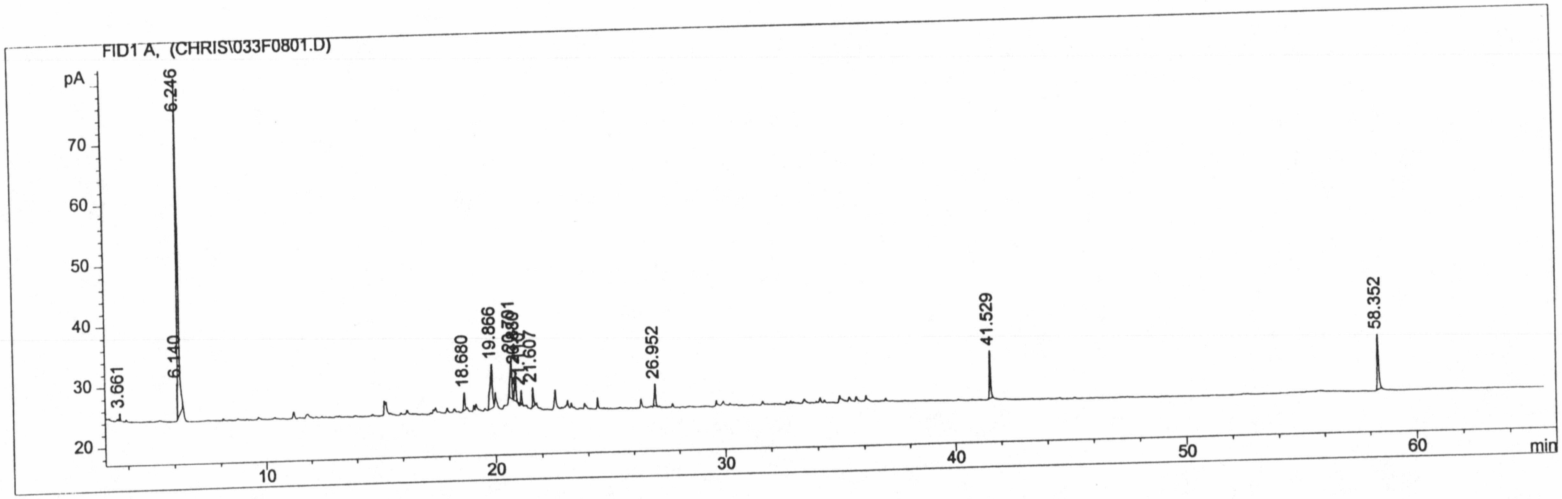
APPENDIX B

GAS CHROMATOGRAPHY (Flame Ionization Detector) CHROMATOGRAPHS

Young Male
(Acidic Fraction)

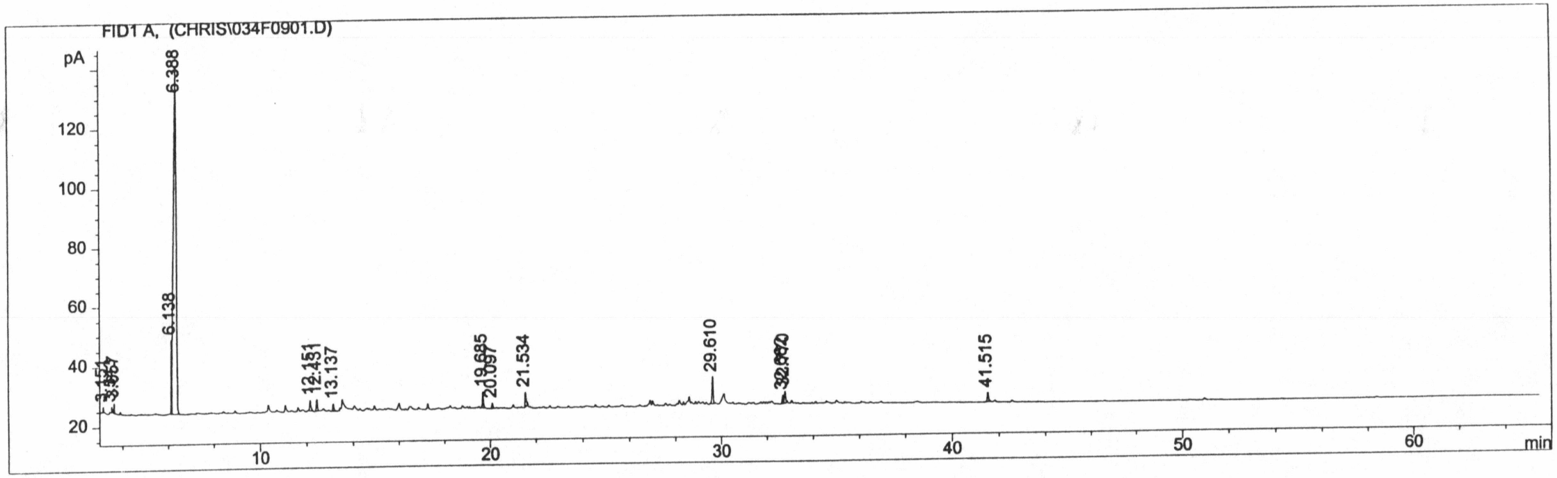


Young Male
(Neutral Fraction)



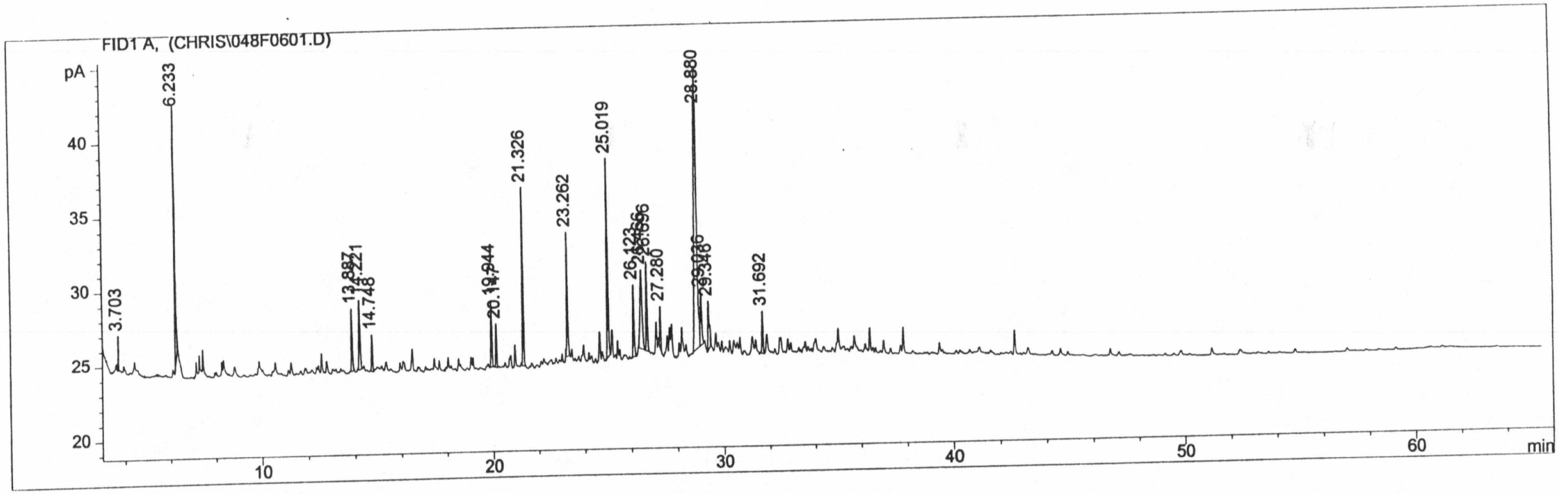
Young Male

(Basic Fraction)

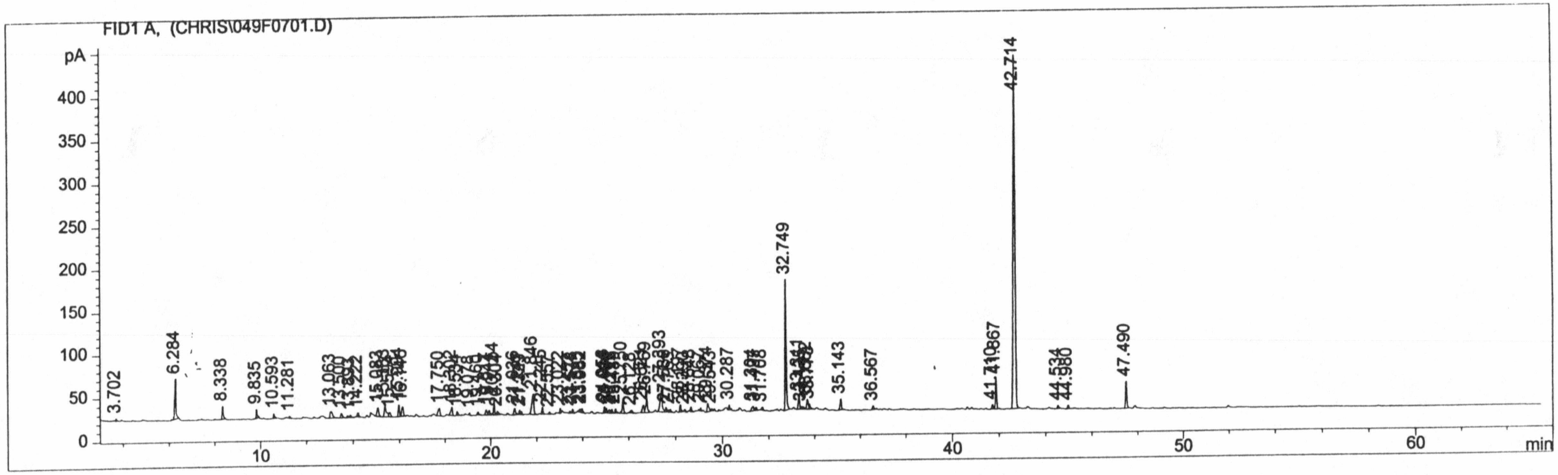


Female Young

(Acidic Fraction)

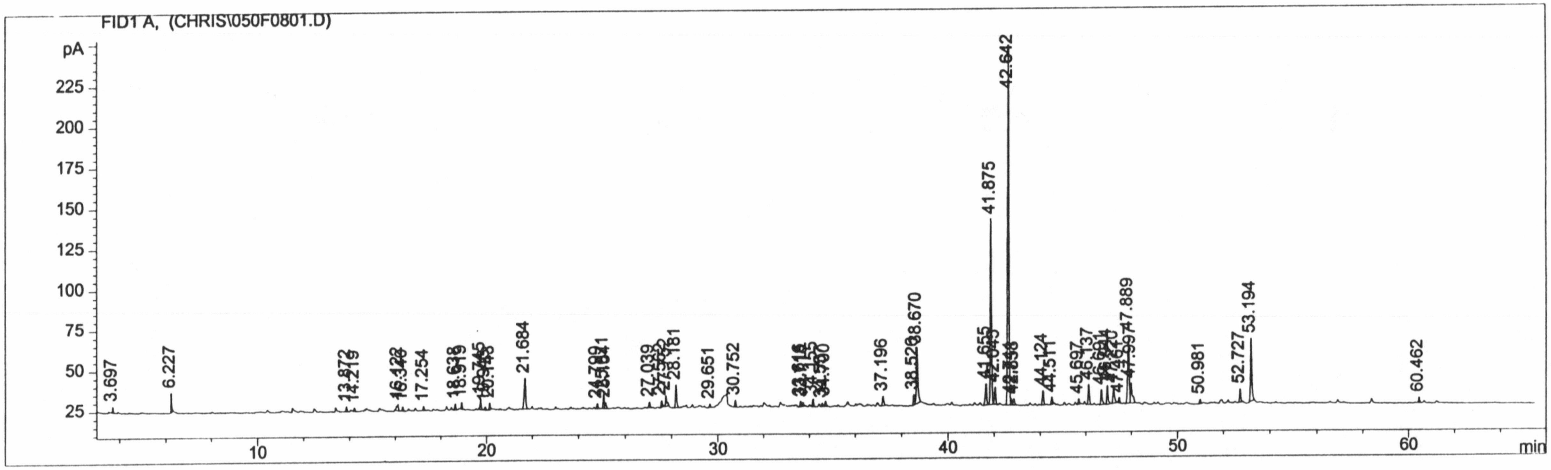


Female Young
(Neutral Fraction)

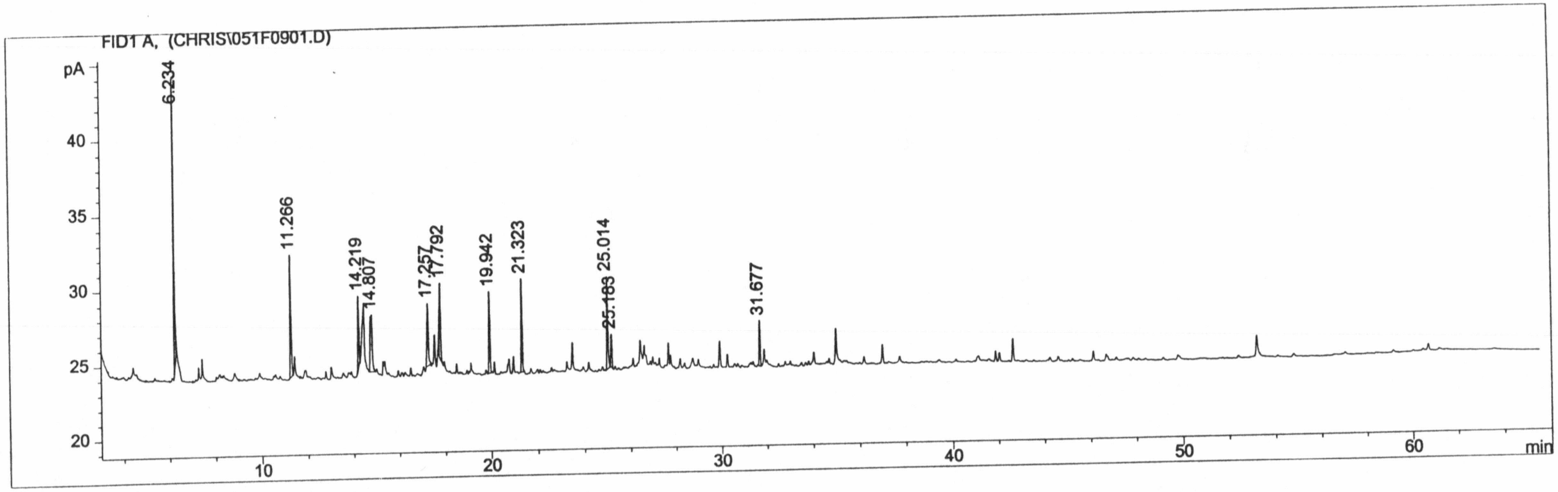


Female Young

(Basic Fraction)

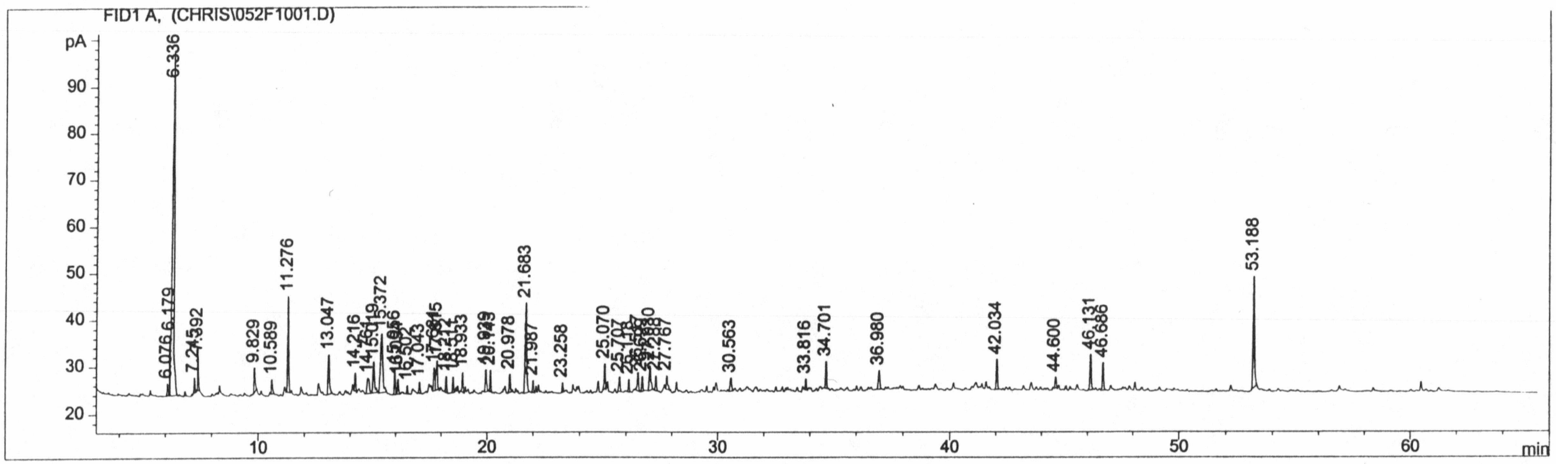


Subadult Male
(Acidic Fraction)

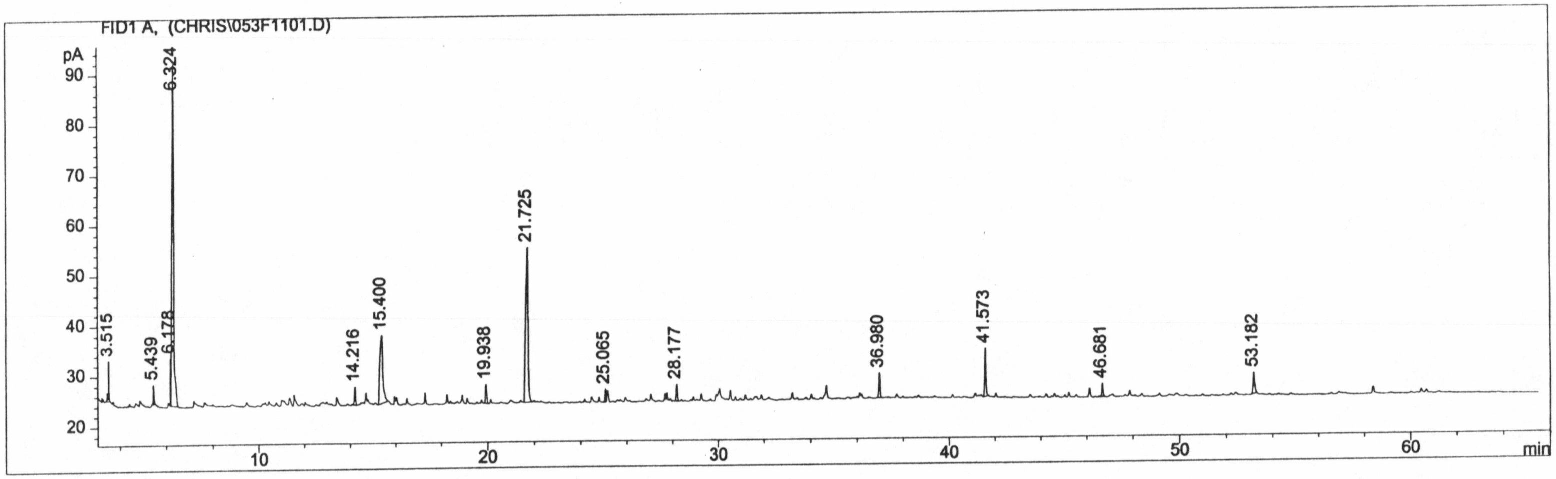


Subadult Male

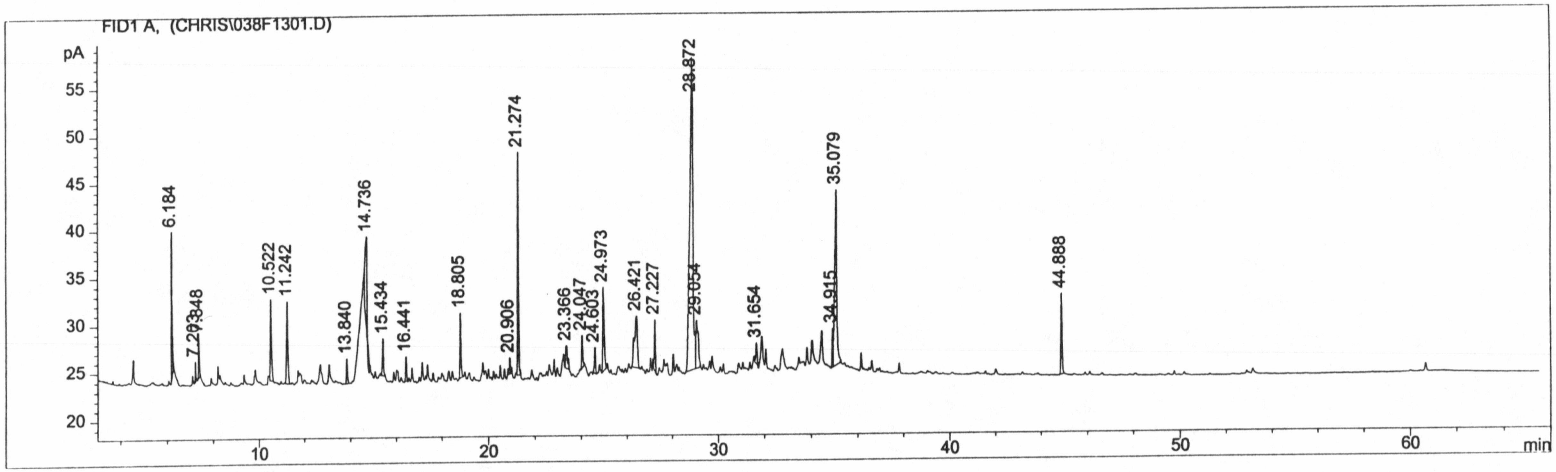
(Neutral Fraction)



Subadult Male
(Basic Fraction)

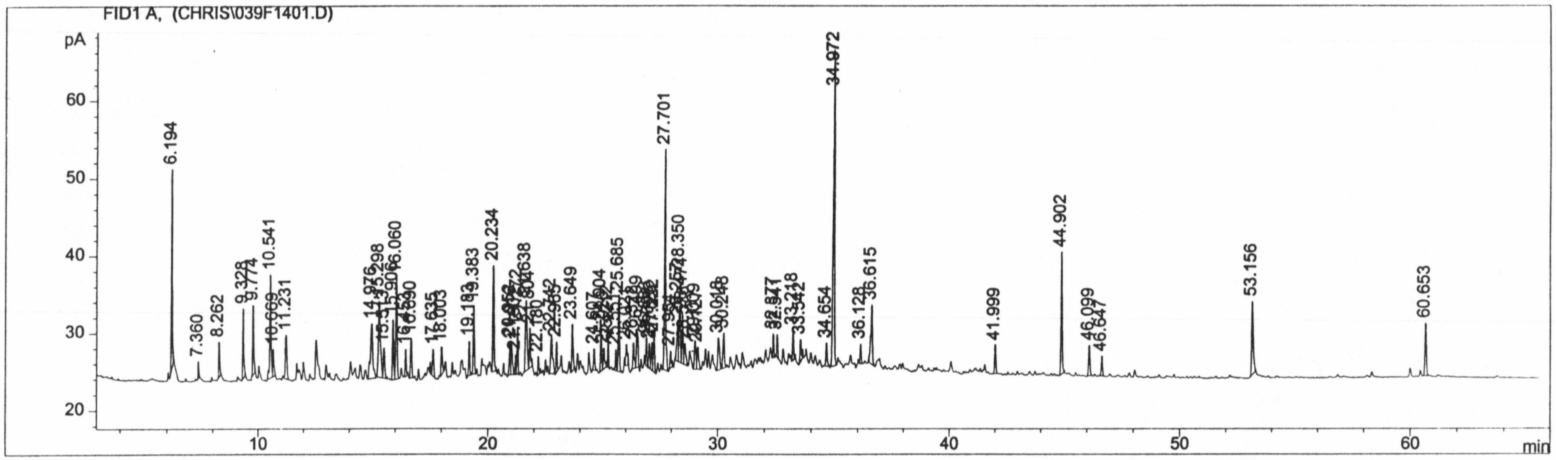


Adult Male #1 - Nonrut
(Acidic Fraction)

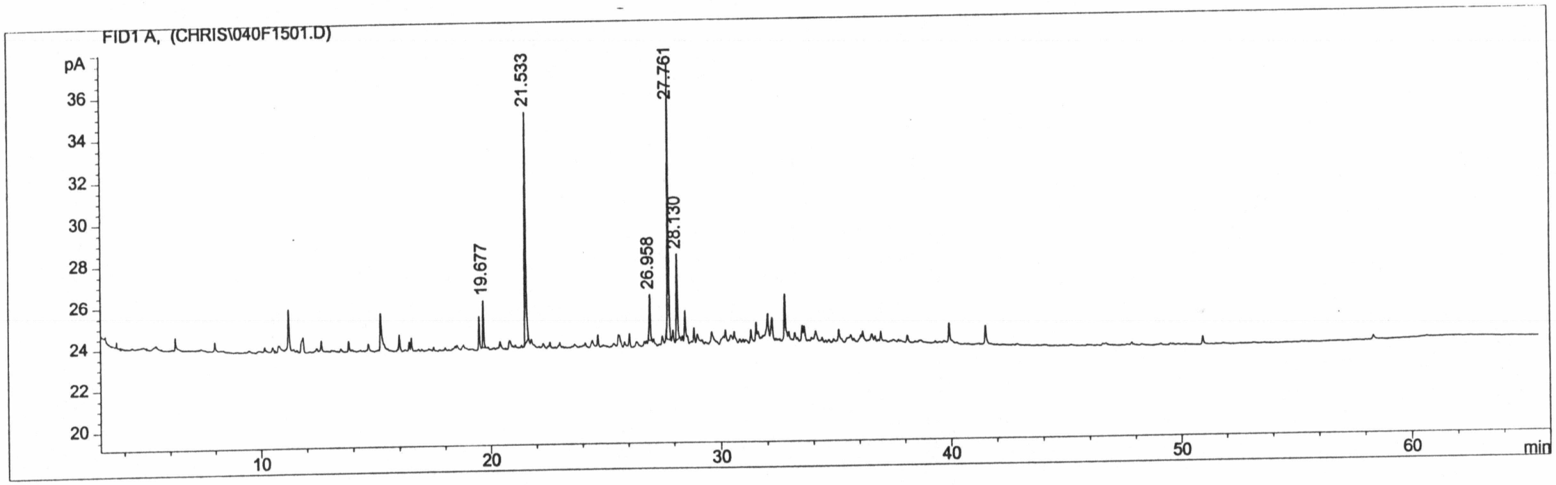


Adult Male #1 - Nonrut

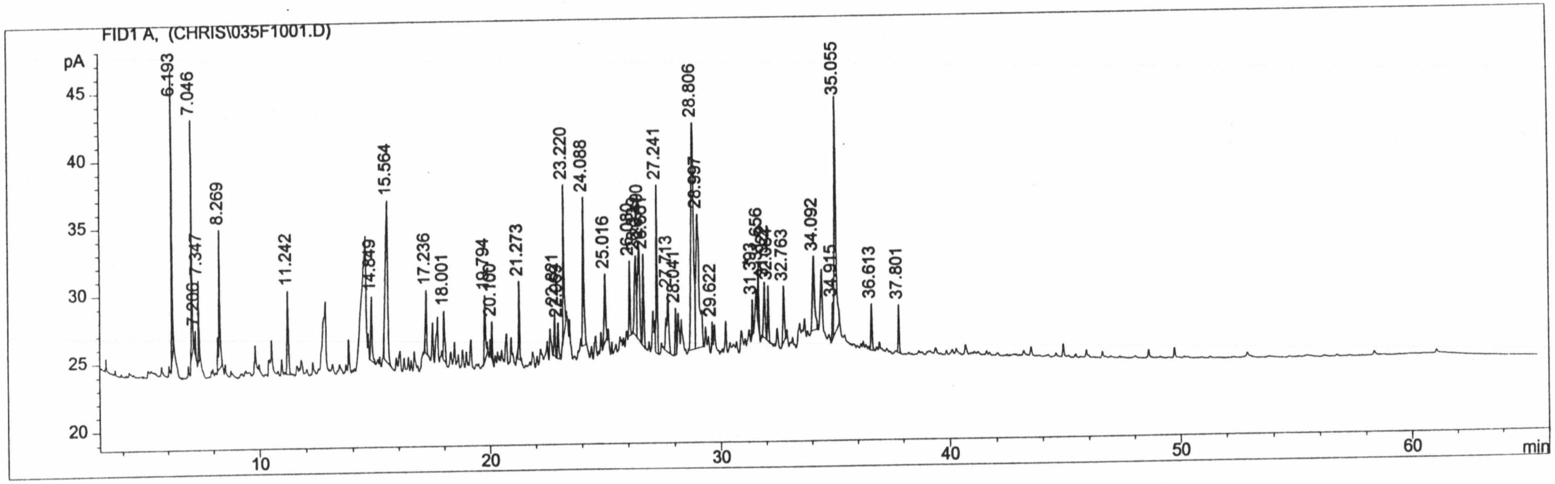
(Neutral Fraction)



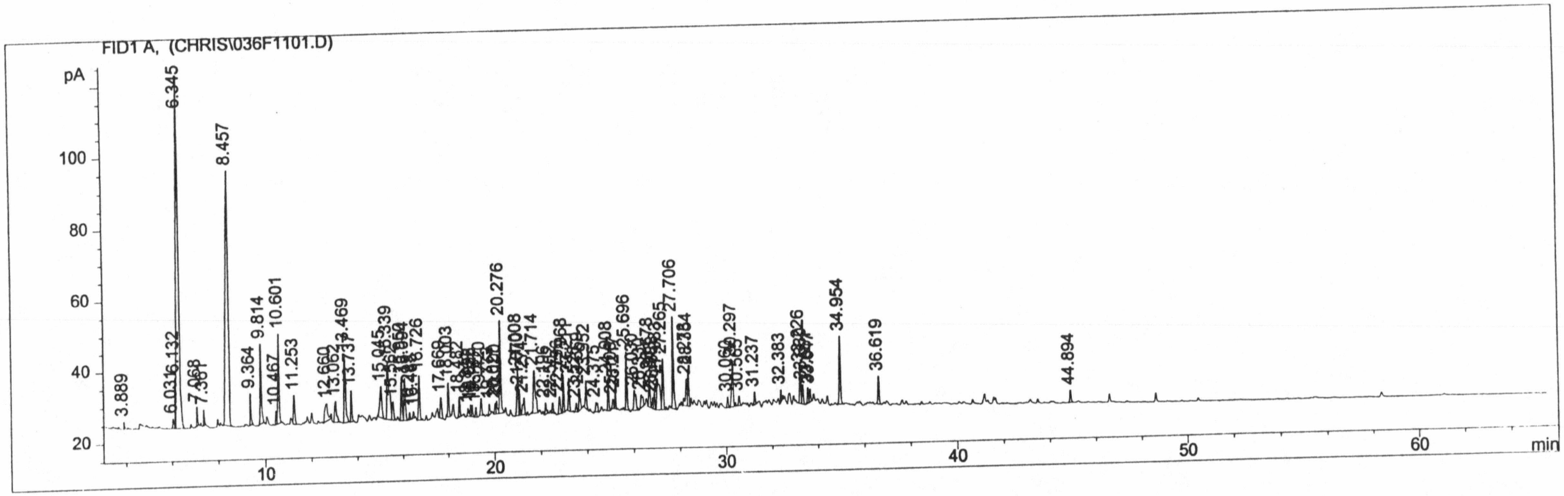
Adult Male #1 - Nonrut
(Basic Fraction)



Adult Male #2 - Nonrut
(Acidic Fraction)

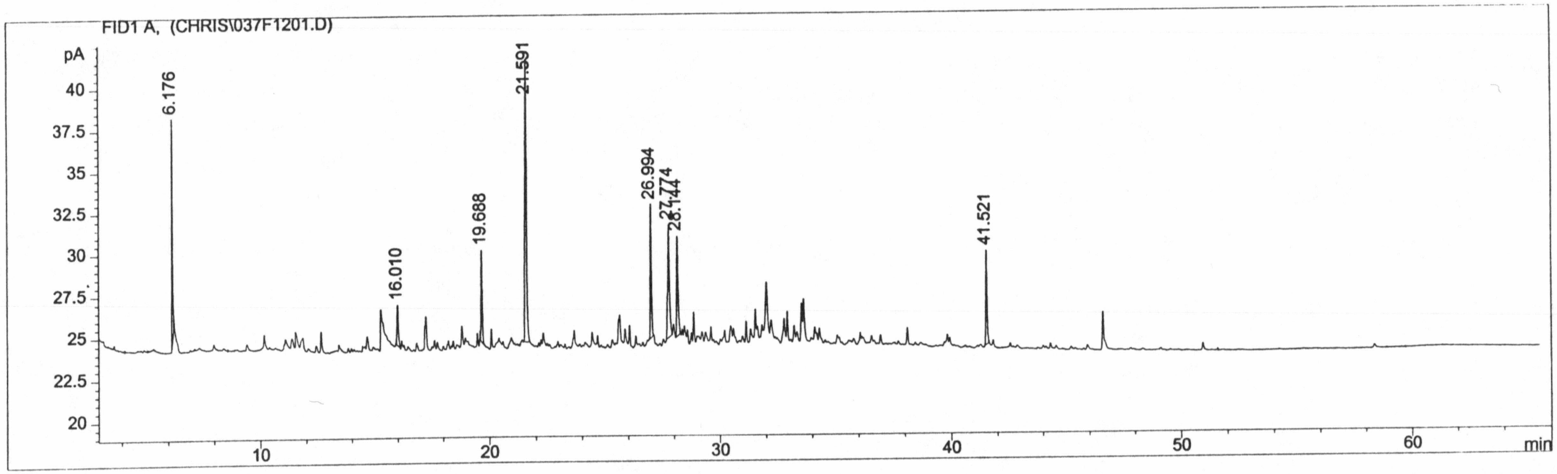


Adult Male #2 - Nonrut
(Neutral Fraction)

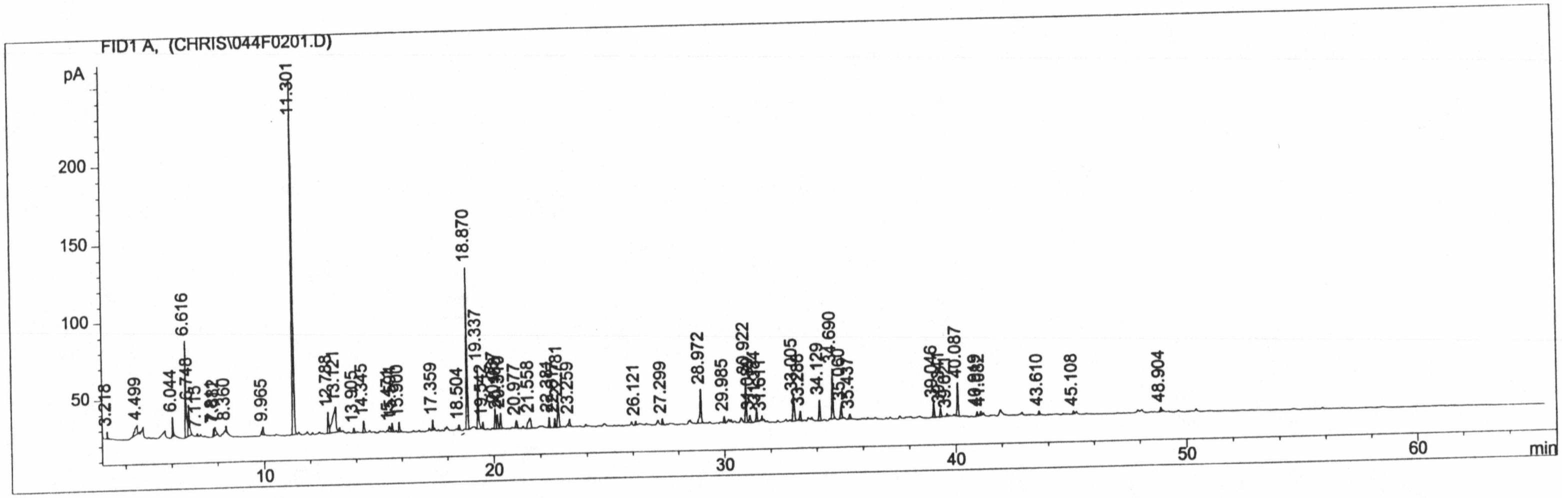


Adult Male #2 - Nonrut

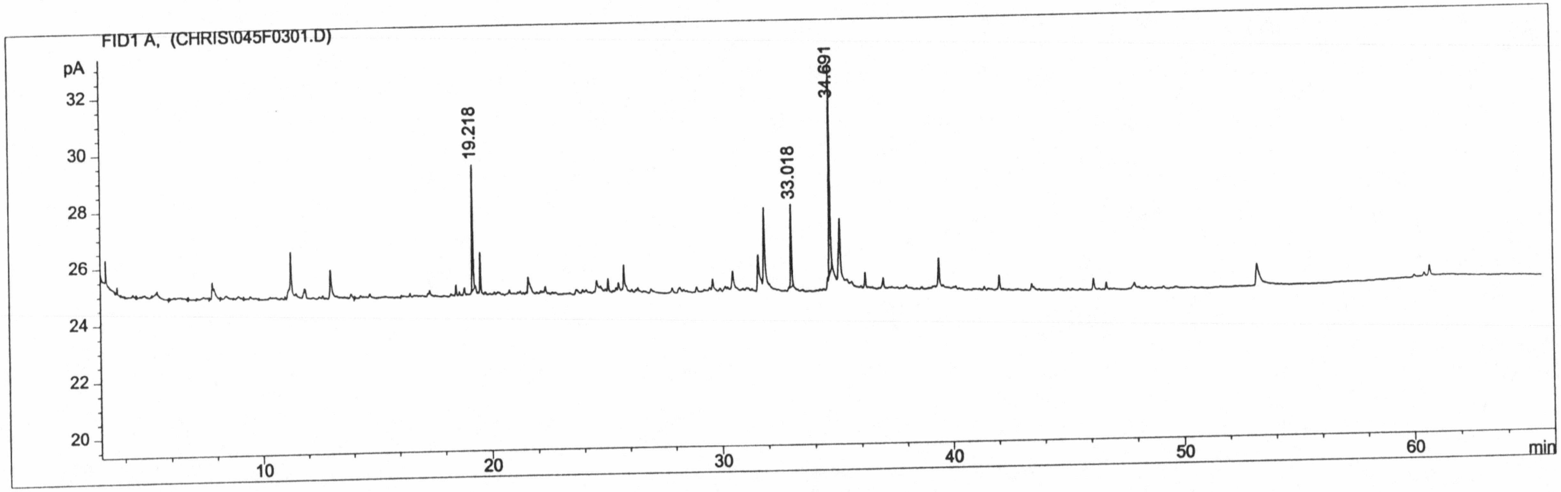
(Basic Fraction)



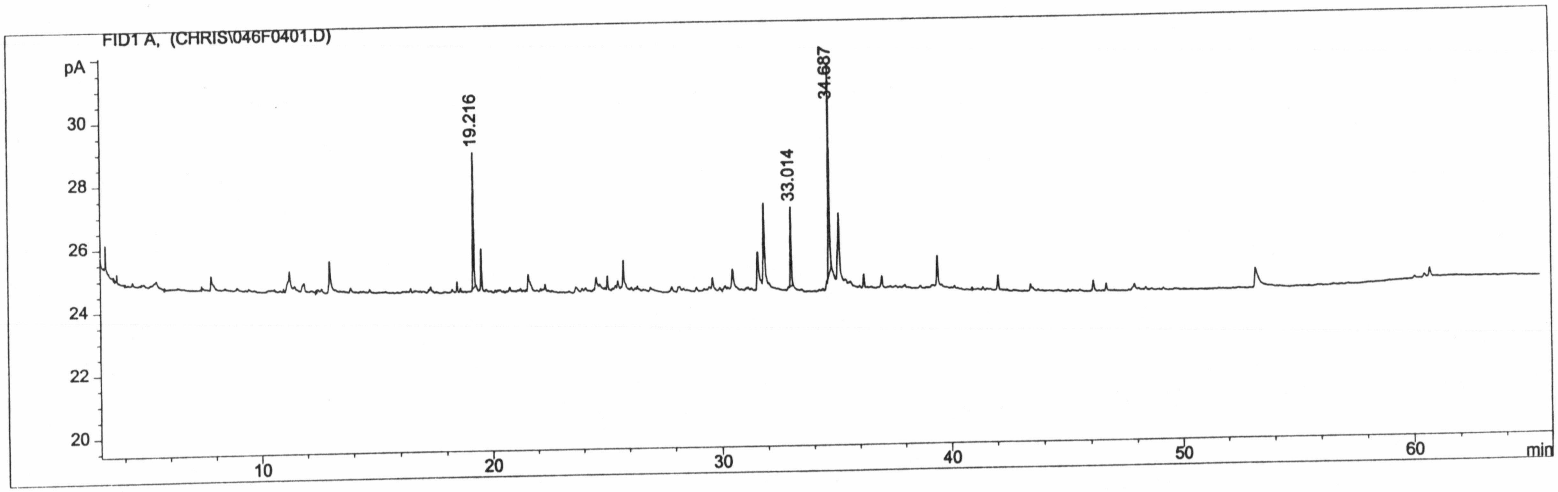
Adult Male #1 - Rut
(Acidic Fraction)



Adult Male #1 - Rut
(Neutral Fraction)

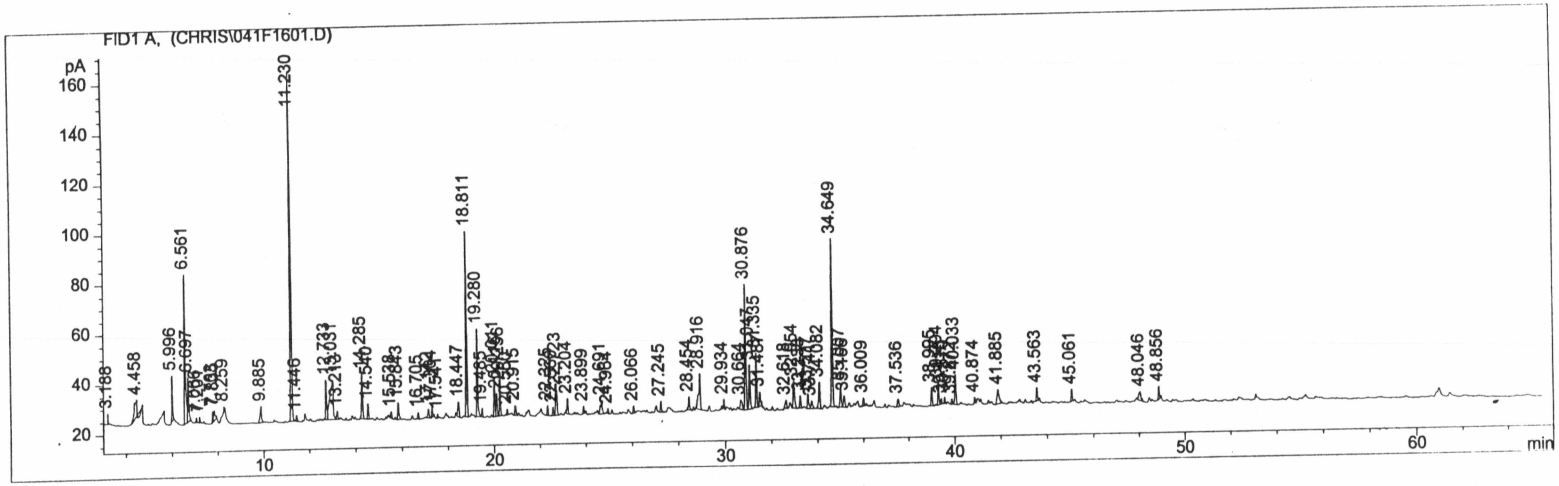


Adult Male #1 - Rut
(Basic Fraction)

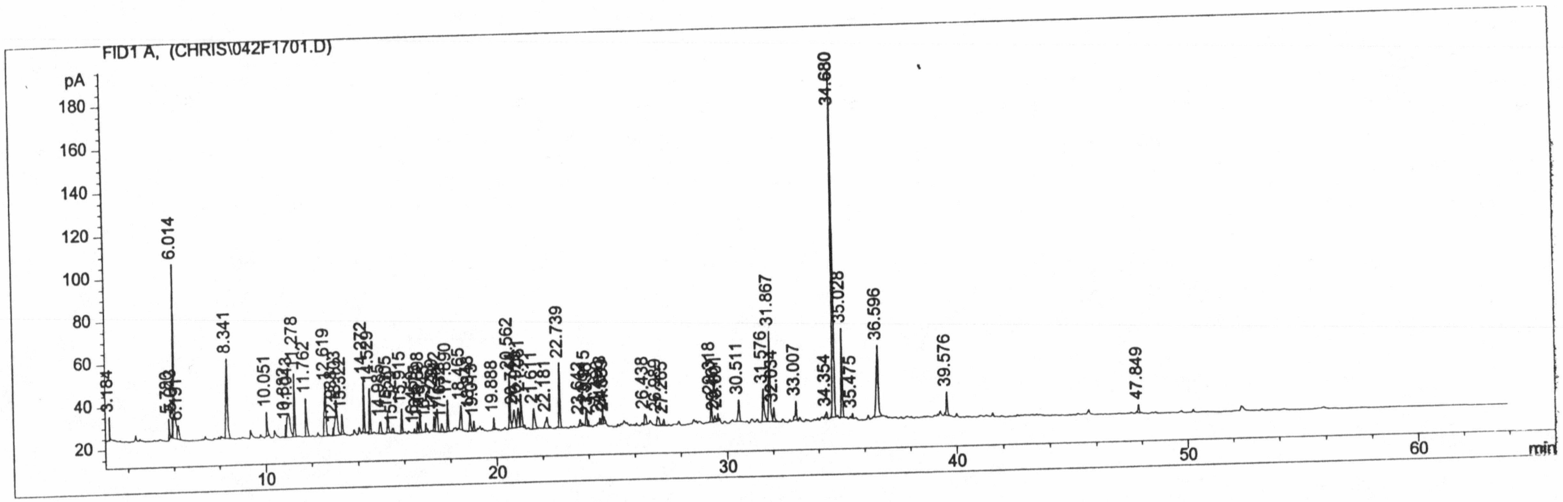


Adult Male #2 - Rut

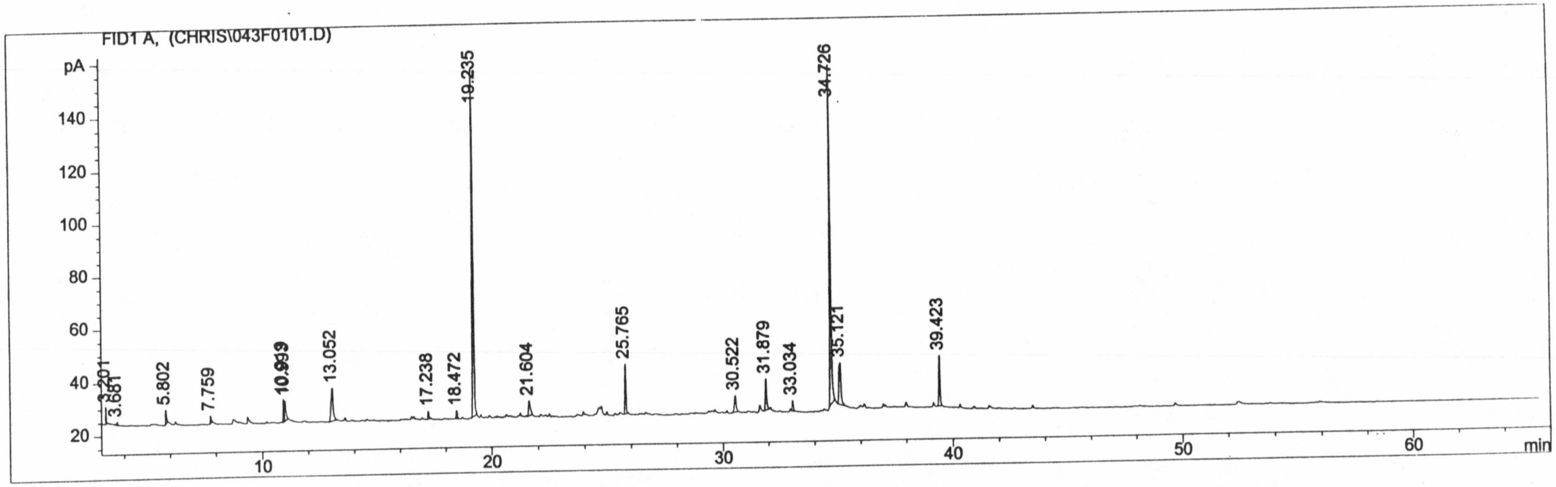
(Acidic Fraction)



Adult Male #2 - Rut
(Neutral Fraction)



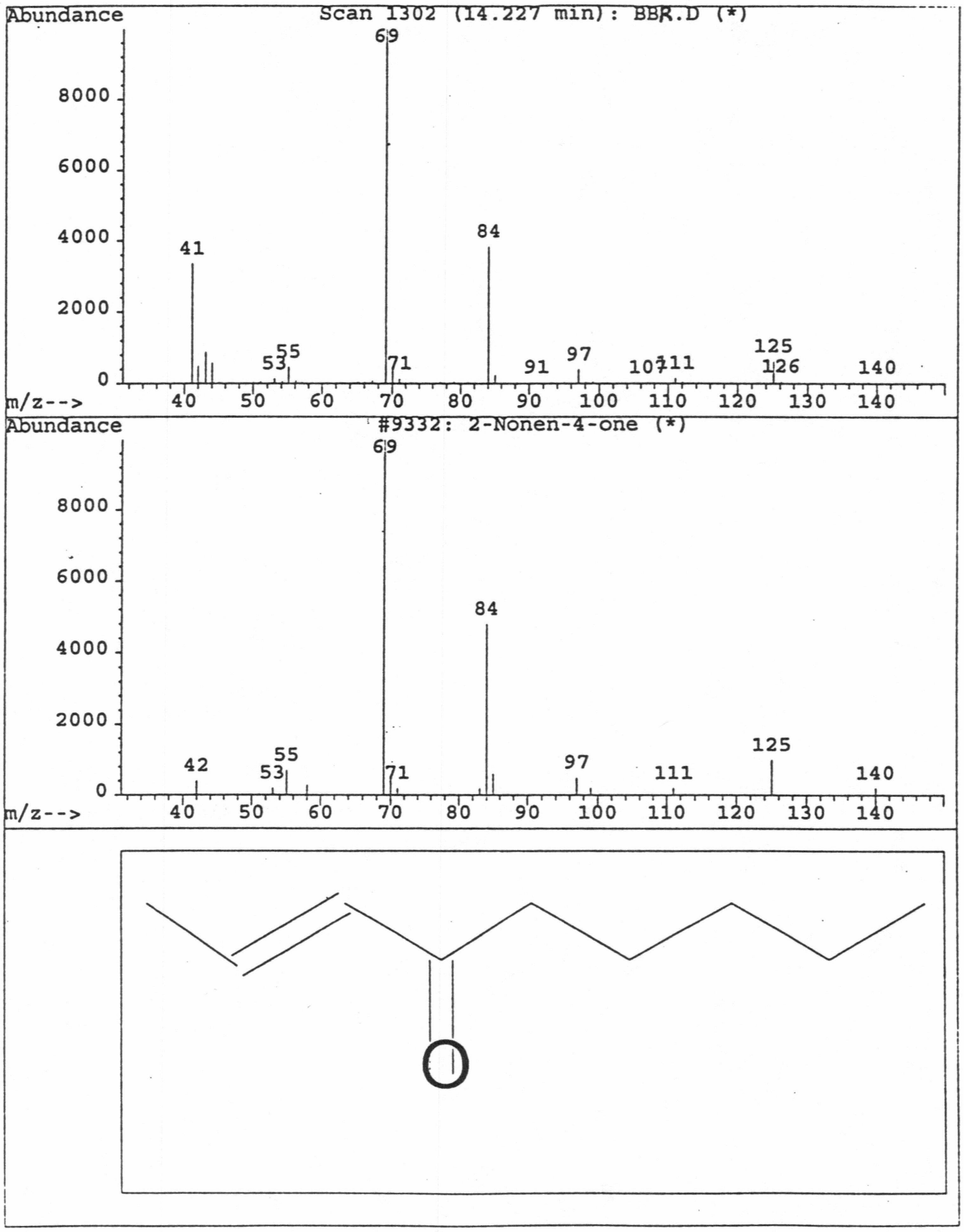
Adult Male #2 - Rut
(Basic Fraction)



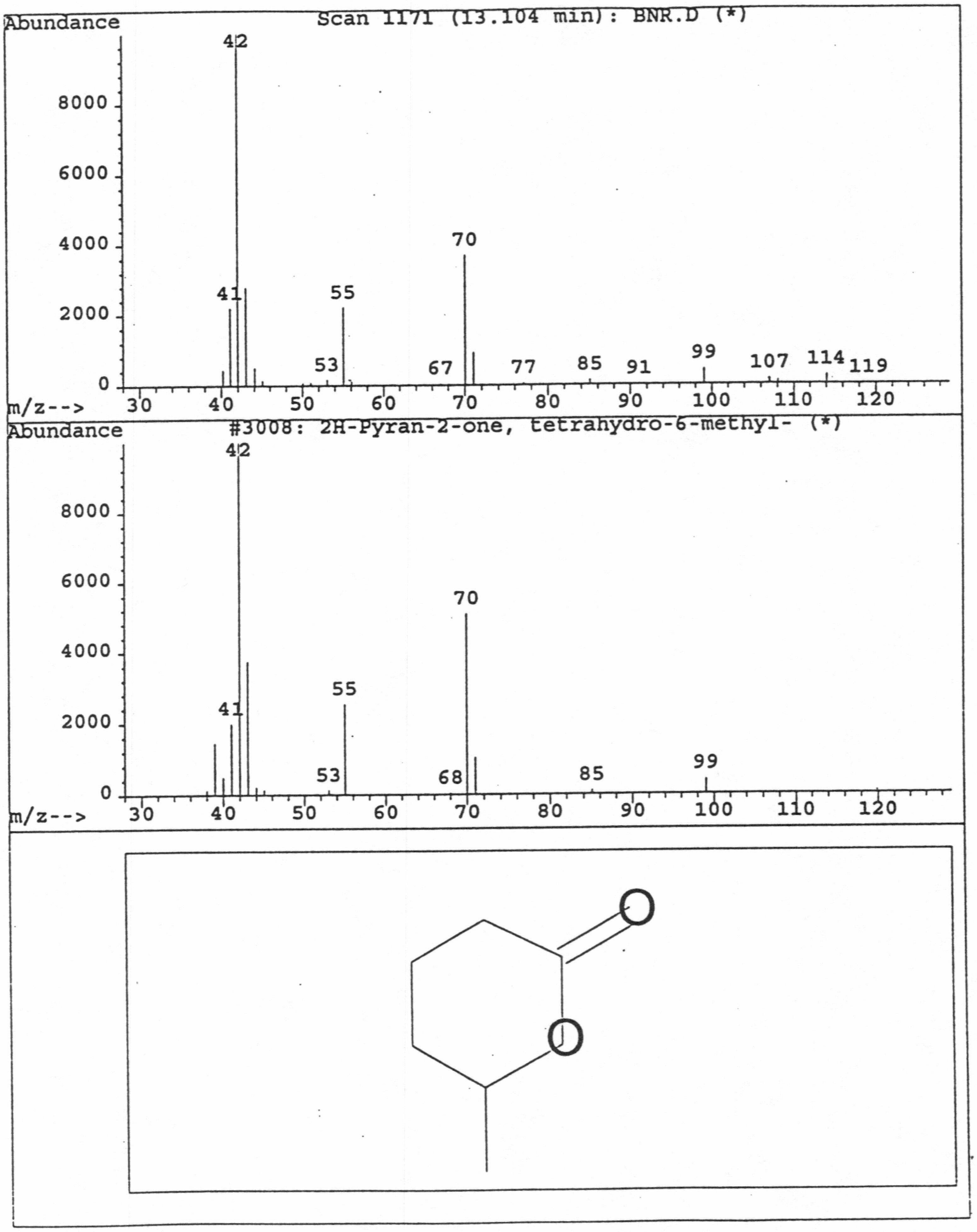
APPENDIX C

MASS SPECTRA OF URINARY COMPOUNDS UNIQUE TO ADULT MALE MOOSE DURING RUT

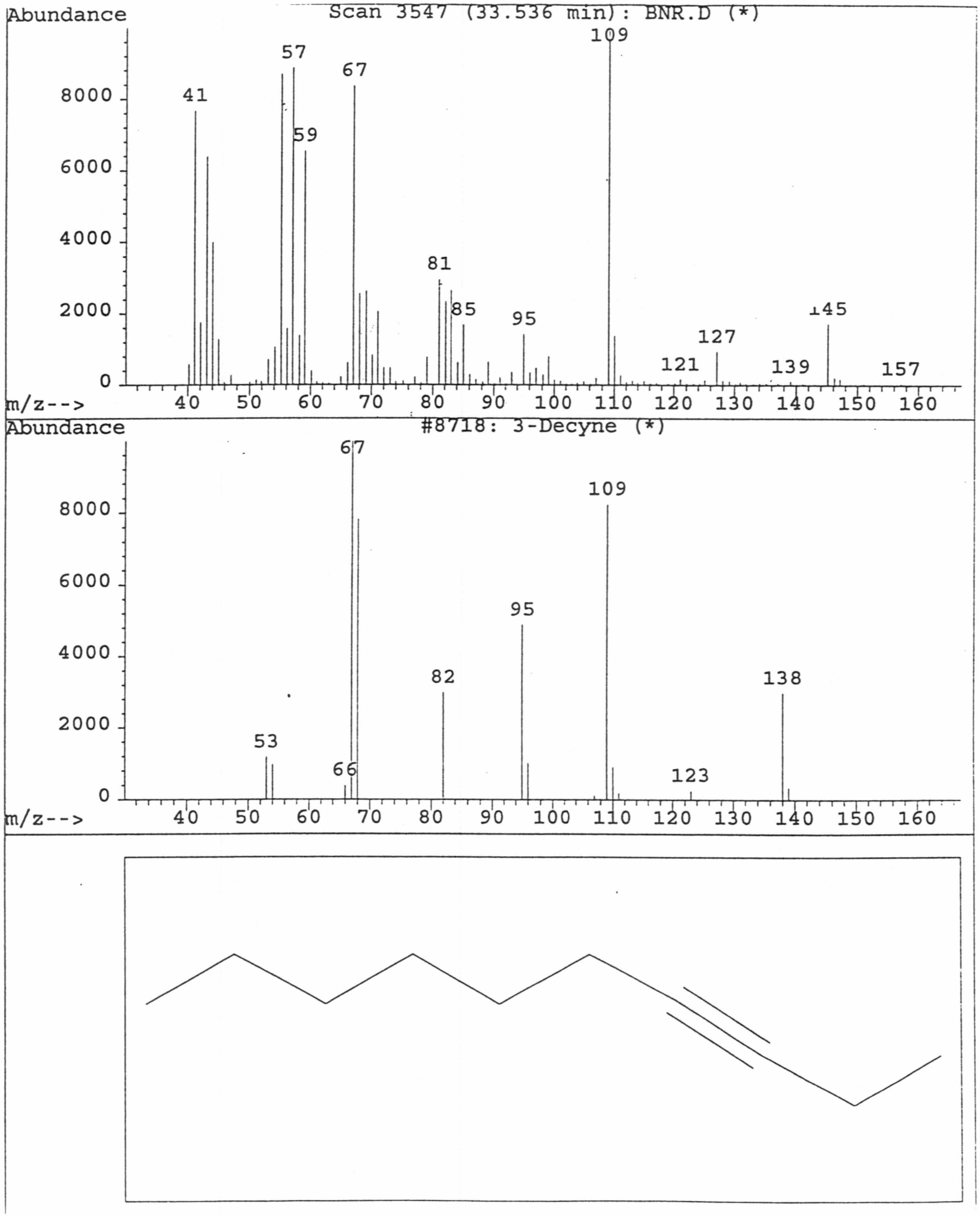
Homolog of 2-nonen-4-one



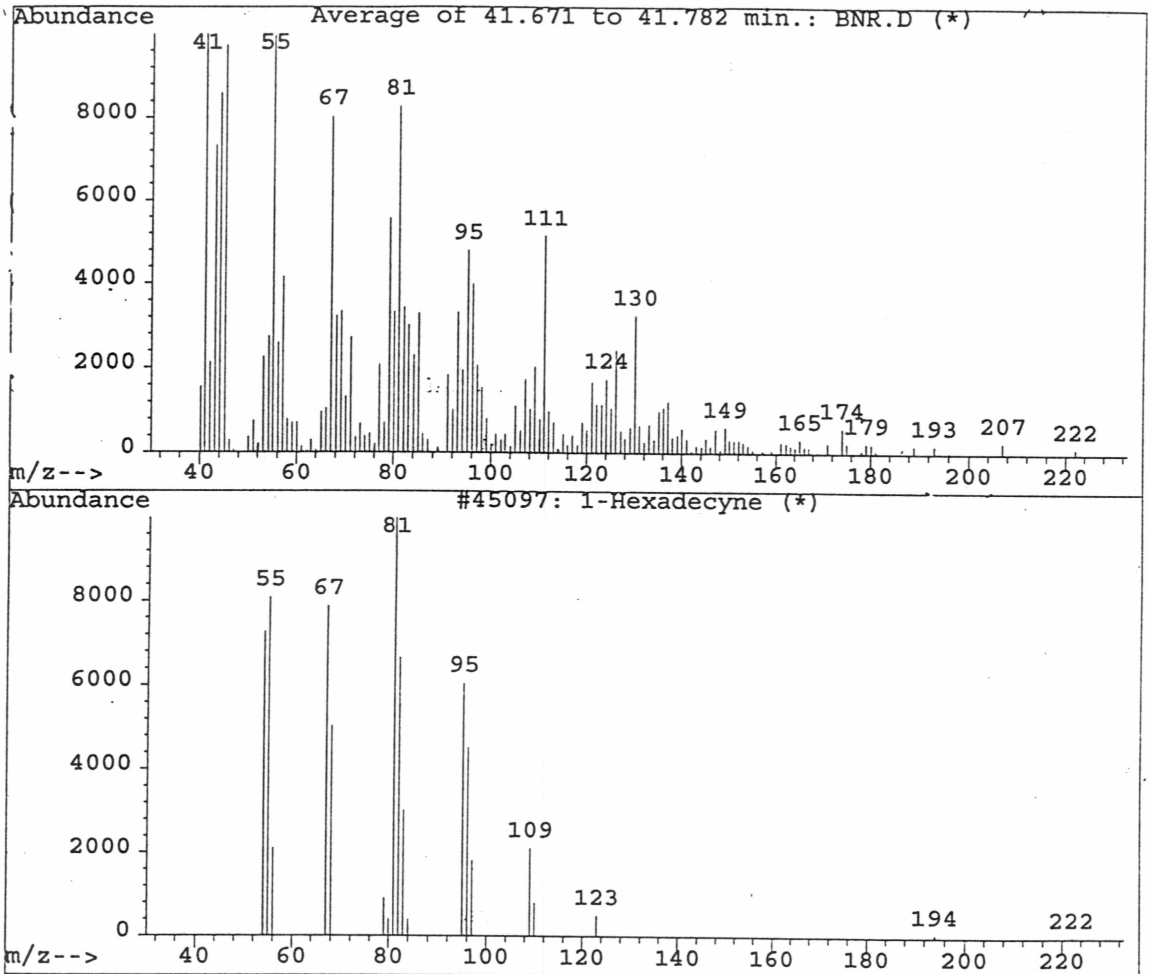
Homolog of 2H-pyran-2-one, tetrahydro-6-methyl

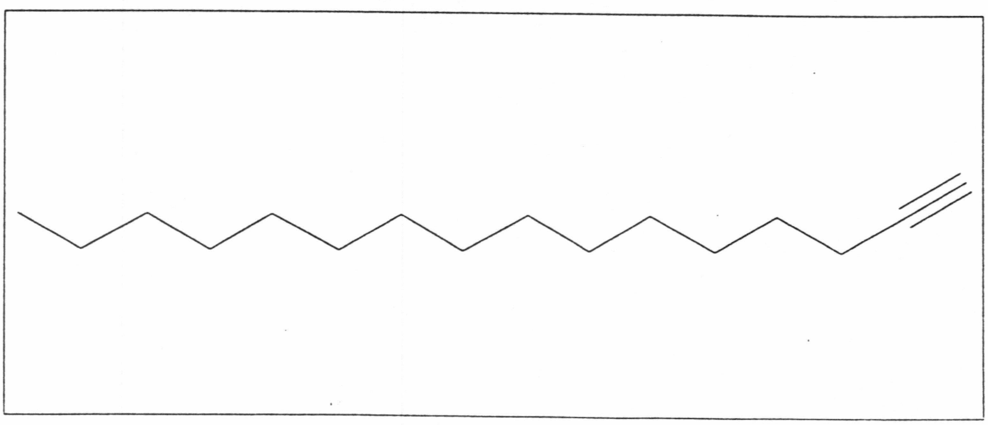


Unsaturated alcohol #1



Unsaturated alcohol #2

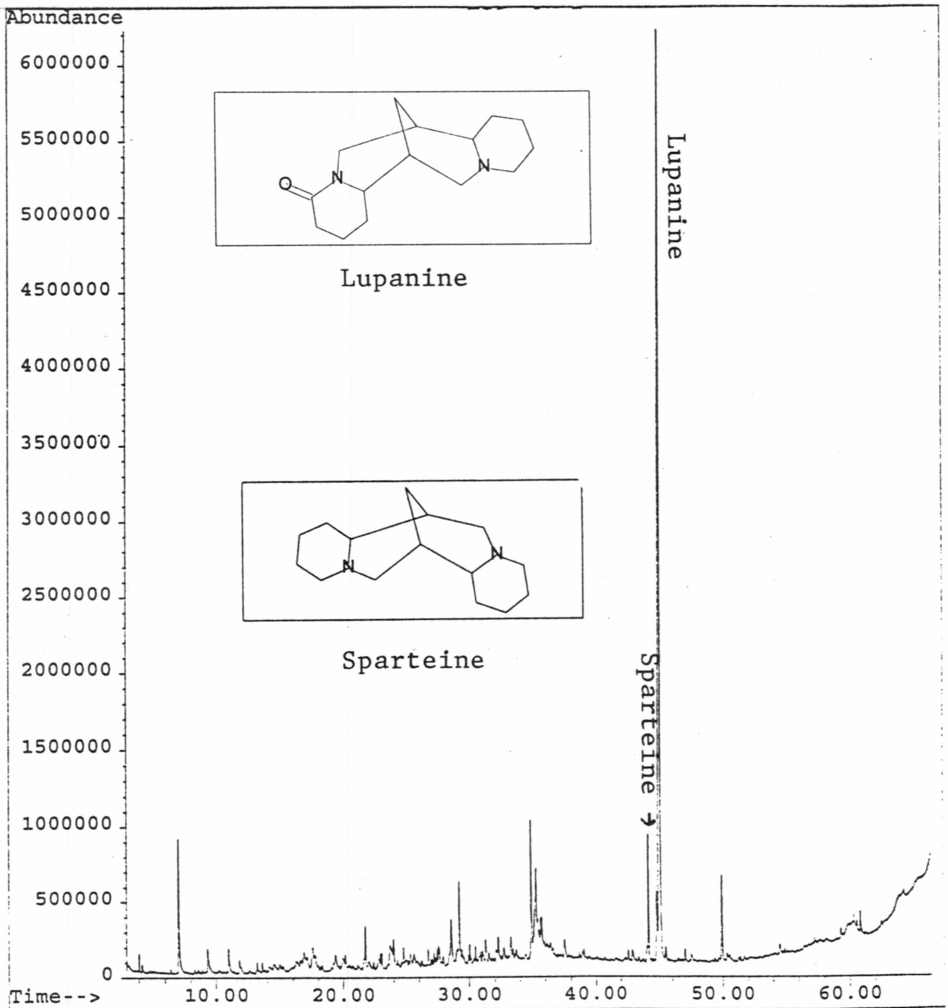




APPENDIX D

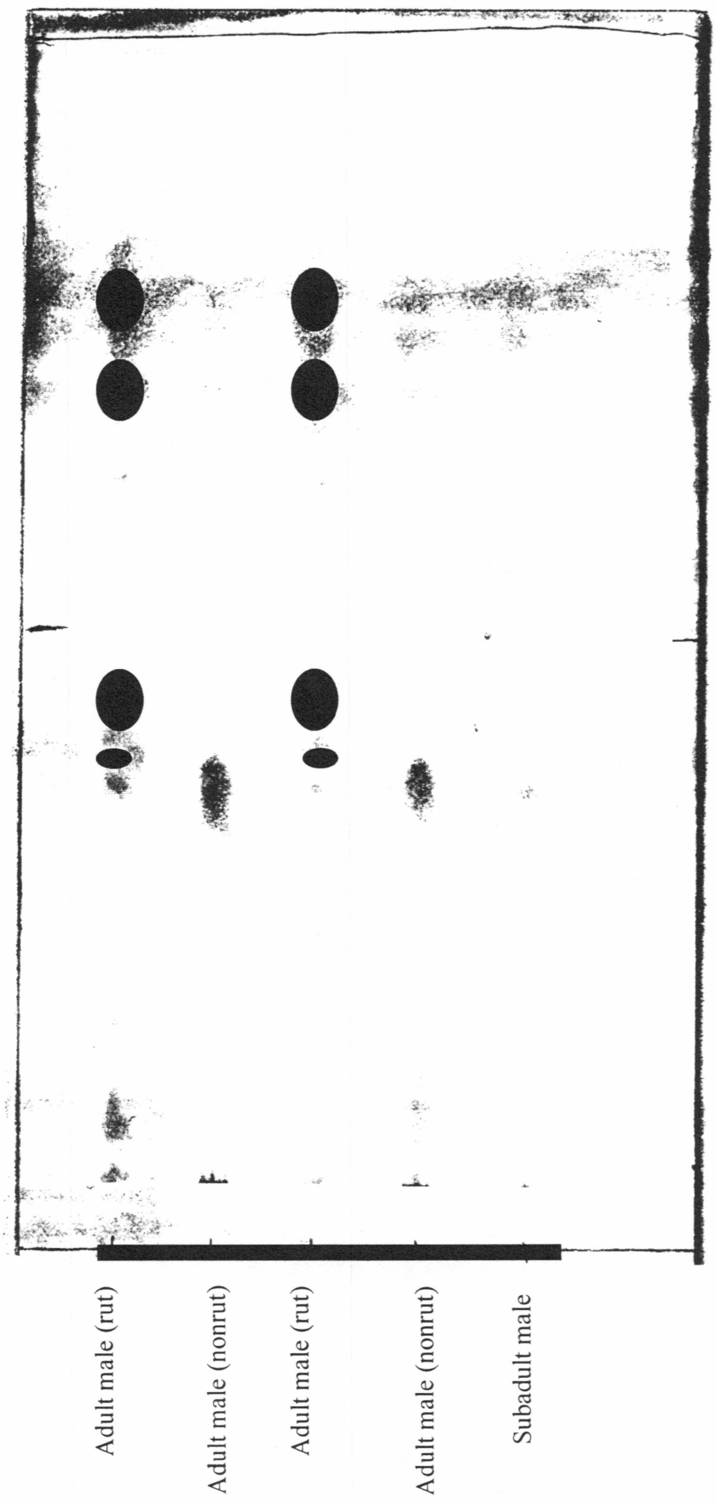
CHROMATOGRAPH OF URINE FROM FEMALE YOUNG MOOSE

(Compounds Identified)

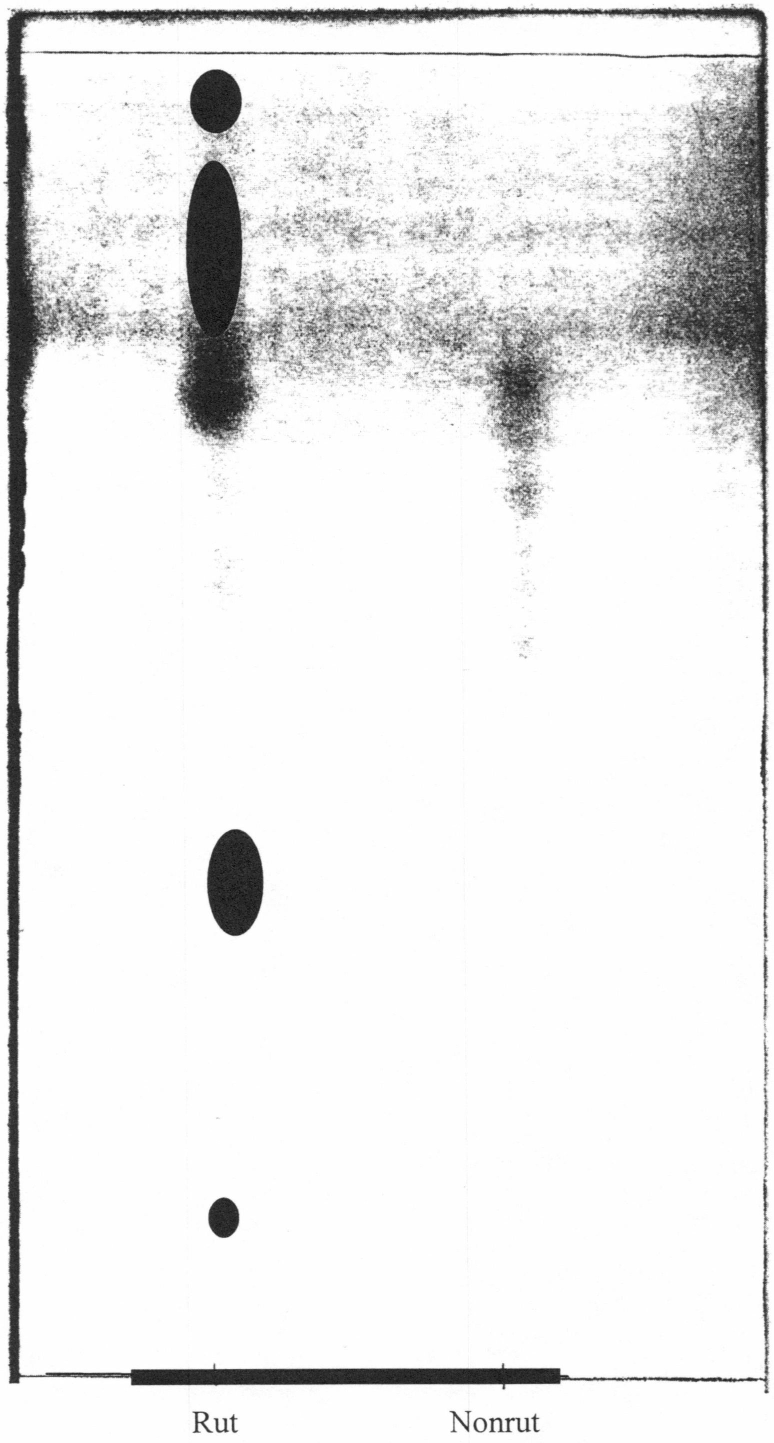


APPENDIX E
THIN-LAYER CHROMATOGRAPHS OF URINE FROM MALE MOOSE

Thin-layer Chromatograph of Urine From Adult Male, and Subadult Male Moose During Rut and Nonrut (acidic fraction)



Thin-layer Chromatograph of Urine From Adult Male During Rut and Nonrut (acidic fraction)



Thin-layer Chromatograph of Urine From Adult Male During Rut and Nonrut (basic fraction)